

UNIT ACTIVITY IN THE HYPOTHALAMUS
AND STRIATUM OF THE RAT DURING
LEARNING

Thesis by

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ABSTRACT

Unit activity was recorded from the hypothalamus and striatum of 80 freely moving rats during an appetitive classical conditioning situation. Responses to auditory stimuli were observed from 118 units before and during a conditioning procedure in which presentation of food occurred one second after the onset of an auditory stimulus. A large proportion of units (111) showed changed responses to the CS during conditioning. Only 8 of these, however, showed new conditioned responses of the very shortest latency measured, 20 msec. after CS onset. These were interpreted as likely sites of rerouting of the stimulus information within the brain as a result of learning. They were located largely near the intersection of hypothalamic and striatal structures. A transient increase in rate of background firing over trials was recorded following the onset of conditioning among hypothalamic units, suggesting they may temporarily represent a dynamic trace of the new learning. No significant differences were found between areas studied in order of appearance over trials of the conditioned responses. However, as a group, the conditioned responses studied here, appeared significantly earlier than a group of cortical neurons studied under similar conditions. There was greater generalization of response to the CS- by units of the basal ganglia than other areas, suggesting they may be of importance in inhibition of response to the CS-. Twenty-three units, located mostly in the

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INTRODUCTION

Psychological theorists have often stressed the importance of motivational and emotional states in the learning of new associations. Hull (1943), for example, hypothesized that an abrupt reduction in a certain drive state following a given learning trial was necessary to the formation of associations between stimuli presented on that trial. Hebb (1955) believed that there was a level of emotional arousal optimal for learning, and that too little or too much was detrimental.

The hypothalamus and adjacent striatal areas of the brain have most often been identified with such motivational functions, both of nonspecific reinforcement (Olds, Travis, and Schwing, 1960; Routtenberg, 1971) and more specific drive-related functions (Hoebel and Teitelbaum, 1962; Anand and Brobeck, 1951; Morgane, 1961; Albert, Storlien, Wood, and Ehman, 1970), though they have not generally been considered sites of learning. There appear to be two possible reasons for this.

First, unlike the cortex or hippocampus, for example, which are characterized by a highly organized internal structure on the basis of which it is appealing to infer circuits within which memories may be stored, the connectivity of the hypothalamus and striatum appears much more diffuse. Nevertheless, cells in these areas do have several features which might recommend them for possibly unique associative functions - the convergence of input from exteroceptive stimuli upon them (Dafny and Feldman, 1970); their ability to monitor

the internal environment of the animal by means, for example, of supposed osmoreceptors and glucoreceptors; their intimate relationship to the endocrine control system of the animal; and their association with the extrapyramidal motor system.

A further reason why the hypothalamus and striatum are often overlooked as possible sites of learning is that traditional methods used to study localization of function in the brain, lesion and stimulation techniques, have overwhelmingly pointed to their role in more exclusively, motivational, regulatory and stereotyped motor functions. This is in contrast, for example, to association cortex, to which it has been difficult to impute any specific functions by standard methods, and of which it is thereby appealing to infer that it was "left over" for more plastic learning functions.

Some attempts have, however, been made to assess the role of these structures in learning through use of stimulation and lesion methods. In the case of the hypothalamus, it has been difficult to separate the two functions of learning and motivation. Lesioned animals suffer such debilitating consequences that a fair test of learning or retention is not possible. Olds and Olds (1958) found that periodic intra-trial stimulation (designed to interfere with consolidation of new learning) of hypothalamus and related limbic structures was more detrimental to learning of a maze than stimulation of other structures of the brain. However, this was open to a second interpretation - that the stimulation may not be so much interfering with the memory consolidation in these areas, as reinforcing competing

responses and thus interfering with the performance of the criterion response. This is less true of the rostral striatal areas where lesions have been described which had detrimental effects on retention of previously learned responses (Rosvold and Delgado, 1956; Gambarian, Garabian, Sarkisian and Ganadian, 1971; Thompson, Malin and Hawkins, 1961) and stimulation has had apparent amnesic effects (Rosvold and Delgado, 1956; Wyers and Deadwyler, 1971). The most interesting of the latter have been a series of studies which have shown that even single pulses of stimulation to the caudate following a trial are sufficient to impair learning on that trial.

Lesion and stimulation studies alone, however, do not ensure that an effect is local; it is necessary to record directly from the area under investigation. To date, there have been a small number of studies in which single unit activity in these structures has been recorded during learning (Olds, Mink and Best, 1970; Olds and Hirano, 1970; Travis and Sparks, 1968; and Kotlyar and Yeroschenko, 1971). As is true of other parts of the brain more extensively studied, these have indicated that a large proportion of these cells change with learning. However, in none of these cases, has an attempt been made to separate those changes resulting from primary changes due to learning that are local to the point of recording, from their secondary consequences, new responses appearing following conditioning which result from projections from other portions of the brain in which primary changes have occurred.

The purpose of the present study was to reexamine the role of the hypothalamus and striatum in learning by recording from single units in these structures, employing a strategy earlier described by Olds, Disterhoft, Segal, Kornblith and Hirsh (1972), which attempts to make this distinction. This strategy is based on a connectionist view of the brain, and on the view that learning "involves the rerouting of nerve impulses within the central nervous system" with "new pathways becoming available to incoming excitation" (Burns, 1957). Such points of rerouting, considered to be sites of learning, would appear to show responses with specific spatial and temporal characteristics. That is, they would be expected to occur at a point to which there was previous sensory input, or at most one synapse removed from such a point, and they would be expected to occur sufficiently early in time after the CS presentation so as to be distinguishable from feedback from the performance of the conditioned response and to be distinguishable from projections of responses occurring earlier in other parts of the brain. Accordingly, prior unconditioned responses to the conditioning stimulus were mapped initially during a pseudoconditioning procedure. Additionally, the latency of responses to the CS was measured with a precision believed sufficient to make the desired temporal distinctions. This is not to say that very early latency conditioned responses only are a result of a local change. Longer latency conditioned responses may also result from primary changes due to learning, but it was not, as yet, possible

to identify these.

An attempt was also made to examine the emergence of the new learned responses in these areas over trials. It was then possible to compare the development of these new responses within the context of the entire brain and of the animal's behavior.

Finally, this method also had the advantage, in the case of the hypothalamus, as previous lesion and stimulation studies had not, of being able to distinguish between possible associative and the more exclusively motivational functions.

METHOD

Subjects

The subjects were 80 Holtzman strain, male, albino rats, 90-120 days old at the time of surgery, and weighing 250-300 gm. Each was implanted with eight chronic microelectrodes for extracellular recording aimed variously at sites in hypothalamus, preoptic area, caudate nucleus, internal capsule, globus pallidus and pyriform-amygdaloid areas, according to the atlas of Konig and Klippel (1963). The preoptic area, as distinguished from the hypothalamus proper, was considered to be that part of the medial forebrain bundle falling at or anterior to section A6360 μ ; probes were designated hypothalamic if they fell in this region posterior to that point.

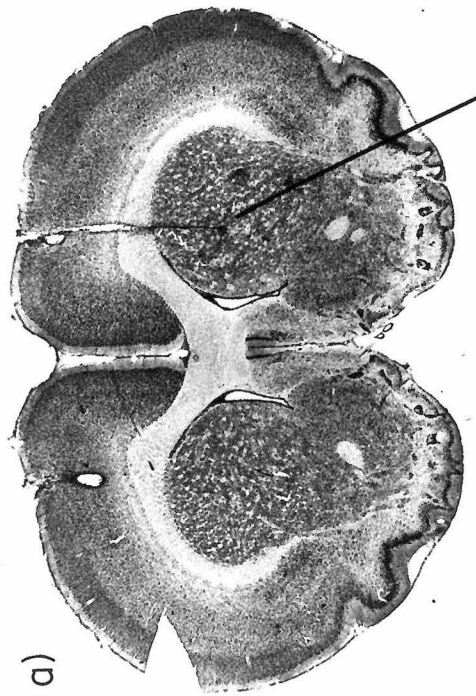
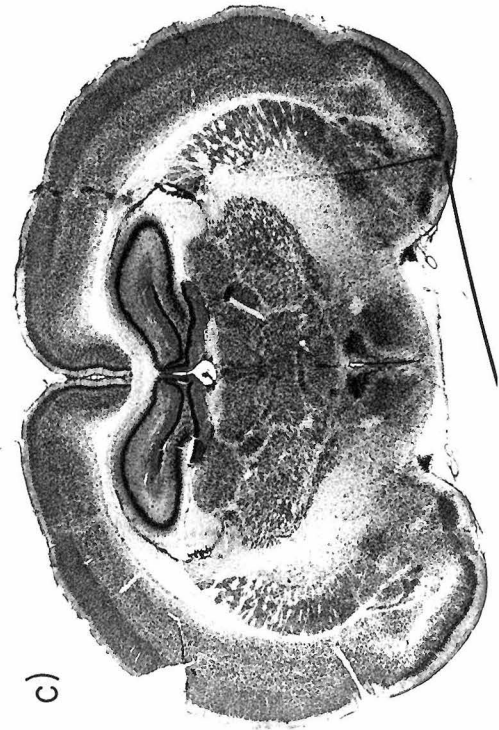
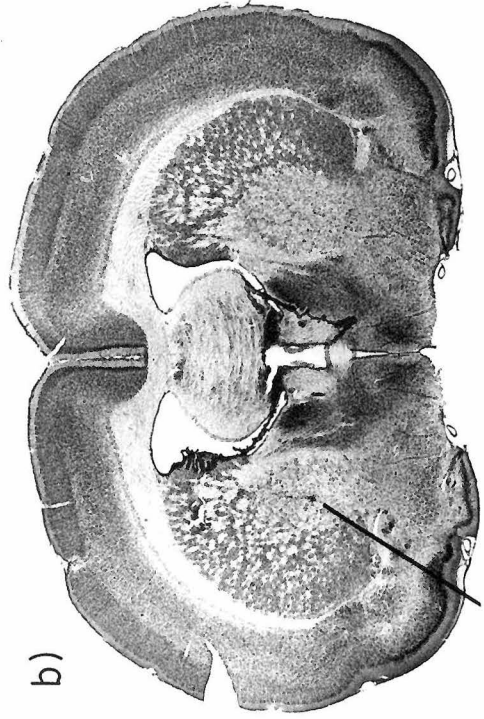
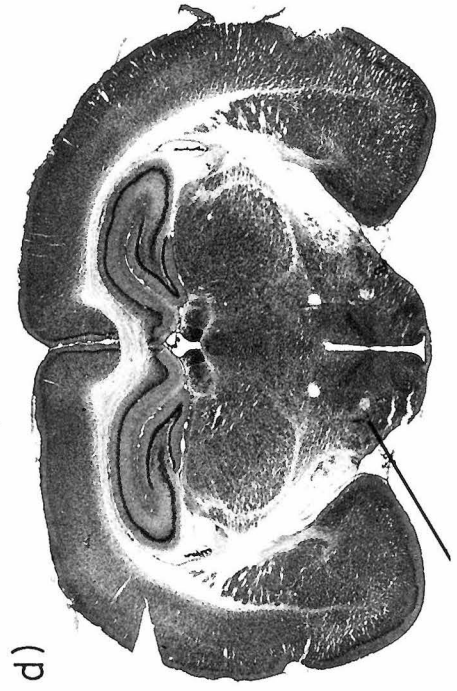
The microelectrodes were made of insulated nichrome wire, 62.5 μ in diameter, cut with a scissors to expose a blunt uninsulated tip. During implantation, unit activity at the end of the probe was continuously monitored. The probe was lowered by a micromanipulator, and was finally fixed when it reached the approximate desired depth, and, if possible, when clear unitary spikes of at least 4 to 1 signal to noise ratio were observed. The background noise level was approximately 25 μ v. and the amplitude of single spikes, 100 μ v. or greater. An additional electrode of uninsulated 250 μ diameter wire, 6 mm in length, was implanted more posteriorly in the animal's brain, generally in the area of the reticular formation, and this electrode served as an indifferent

against which unit recordings were made. All wires were affixed to the skull with an acrylic cement. They were then threaded into a 10-contact teflon plaque which was similarly cemented into place. The animals were allowed at least 3 days for recovery following which they were subject to periodic screening. Following surgery, they were maintained on a continuous 24-hour light schedule, and on a limited diet of laboratory rat chow, sufficient only to keep them at approximately 80% of their normal body weight. They were chosen for experiment when they showed clearly discriminable unit activity on 2-4 of their electrodes.

Following the two-day experiment, the animals were sacrificed by an intraperitoneal injection of a lethal overdose of nembutal. A marking lesion was made at the tips of those electrodes used in the experiment by passage of a 10 μ a. direct current for a period of 20 sec. The animals were then perfused with physiological saline, followed by a 10% formalin solution. The brains were removed and subjected to standard histological procedures. Sections of 60 μ diameter were alternately stained for cells and fibres by cresyl violet and Weil techniques respectively. The sections were then examined under magnification to determine the exact location of the electrode tips. Examples of such histological sections are shown in Fig. 1.

Figure 1.

Sample histological sections following cresyl violet stain.
Probe tips are located in a) caudate nucleus, b) globus pallidus,
c) pyriform cortex and d) hypothalamus.



Apparatus

During the experiment, the rats were placed in round plexiglass cages, 13 inches in diameter, which were further enclosed within a larger sound attenuating chamber. Each cage was equipped with a loudspeaker for presentation of the tone CS, a pellet dispenser for delivery of the 45 mgm. food pellet UCS, and a continuously available water bottle. The cage was also dimly illuminated. An 11-wire cable penetrated through the center of the top of the cage. This was affixed at the lower end to the animal's plaque, and at the upper end, to a commutator and counterbalanced arm to allow the animal relative freedom of movement. Of the 11 wires, 10 were low noise cable and were connected to the animal's electrodes; the eleventh was a high noise length of an electrically unterminated cord. Movements of the animal caused large voltages to be generated in this lead. These voltages were fed through an amplifier with a frequency range of 500-2000 Herz and then into a Schmitt trigger; the trigger output was then used as a measure of the behavioral response of the animal. The trigger level was preset for each animal at the beginning of each experiment such as to allow minimal movements of the animal to be recorded.

Electrical signals from those electrodes chosen for recording during the experiment were fed through amplifiers with frequency range 500-10,000 Herz and then into waveform discriminators which

utilized spike height and time constant "windows" to select single units for counting. Spikes that appeared on a storage scope to be from several different cells were often so similar in amplitude and wave-shape as to be indistinguishable by the discriminators. Nevertheless, each spike was in itself believed to be one action potential from one neuron, and all the neurons contributing to the group recorded as one "unit" were believed to be from a small family of similar neurons localized at the recording point. Samples of the wave-shapes being selected by the discriminator were drawn out by the computer in approximately 30 sets of 10 overlapping traces (300 units) over the course of the experimental sessions. The quality of these drawings was evaluated at the end of the experiment, and if clearly non-unitary spikes were included in the data, or if spikes of widely different amplitude and wave shape were counted as one, on either day of the experiment, the data were discarded. Because electrical artifacts associated with stimulus presentation could have added false positive cases of short latency responses, the exclusion criterion was set at a level which was stronger than necessary. Most of the excluded cases in fact yielded data in substantial agreement with other data from the same brain areas. Sample computer drawings of acceptable and unacceptable units are illustrated in Fig. 2.

Figure 2.

Sample computer drawings of units recorded from in this experiment over pseudoconditioning and conditioning days. Data from units of comparable quality to the upper three represented here were accepted; data from units of comparable quality to the lower set of traces was not accepted.

PSEUDOCONDITIONING
DAY 1

CONDITIONING
DAY 2

Accepted:



Not accepted:



Procedure

The experiment consisted of two 16-hour sessions run over two successive days, from approximately 4 PM to 8 AM the following morning, separated by approximately an 8-hour interval. During a period of about an hour just prior to the experiment the first day, the animal was handshaped to respond to the sound of the feeder by retrieval of the pellet. This was the first experience the rat had with a feeder and pellets of this kind. It was given 20 or 30 such trials until the latency of this response was in the order of a few seconds. The sound of the feeder then served as the UCS in the actual experiment. During the experiment, pellets not retrieved within seven seconds following delivery were automatically withdrawn. The pellet withdrawal was also accompanied by a brief auditory stimulus. The number of pellets missed by the animal was counted each morning, and if the animal failed to retrieve at least two-thirds of the total given on the conditioning day (a measure of the effectiveness of the UCS) the data were discarded. Following shaping, the programmed experimental procedure of pseudoconditioning and habituation was begun. Approximately 300 trials of each of the three stimuli, CS+ (one tone), CS- (the second tone), and the feeder alone, were presented to the animal in pseudorandom order at a mean interval of approximately one minute. The two tone stimuli were auditory signals of 1000 c.p.s. or 10,000 c.p.s. square pulses

of two seconds train duration. The low tone was randomly assigned as the CS+ for approximately one-half of the animals, the high tone, for the remaining animals.

The second day was primarily devoted to conditioning. However, the pseudoconditioning procedure was retained for the first two hours (120 trials of approximately 40 of each kind). A switch was then made to the conditioning series without any other break in procedure. During conditioning, there were similarly approximately 300 CS+ and CS- trials. The CS+, however, was followed by the UCS of the feeder at an interval of one second. Thus the tone overlapped the feeder presentation in the last second of the trial. The CS- continued to be presented singly. A third trial category, the blank trial, was substituted for the feeder trial of the first night to maintain a comparable temporal distribution of trials to the pseudoconditioning night. There were thus an equal number of CS+, CS-, and pellet presentations during the 16-hour period during both the pseudoconditioning and conditioning sessions.

Spike data were collected over a 3-second interval on each trial. Recording began one second prior to the first stimulus presentation (CS+ or CS-), without any sign to the animal that the trial had started, and continued for three seconds. The first second was taken as a measure of the background activity; the following second indicated the response to the stimulus. The data from the

third second were taken with a view to studying correlates of feeding, but the results were inconclusive.

Time Intervals

Within each trial the minimum analysis time division (bin) was 10 msec; thus there were 100 divisions per second. Finer grain analysis of latencies therefore could not be made. On each trial (for each probe) a 1 or a 0 was placed in each minimum time bin depending on whether there had been any unit identifications made during that time interval. While it would have been more accurate to place the actual counts in these bins, critical evaluation of preliminary data showed that the same latencies and the same curve shapes were generated by either method. This was probably due to the low probability of firing in the units studied. Therefore, because binary entries were less expensive, this method was chosen.

For each probe, average pre- and post-stimulus histograms were computed for the first and second group of 150 trials on the pseudoconditioning day, and for the 300 trials of the conditioning day. The pseudoconditioning trials were divided into two groups so that the magnitude of the new conditioned response could be compared to the habituated response to the CS on the pseudoconditioning day - that is, to the response recorded during the last 150 trials on that day. In computing averages, the contents of all the first bins were added,

similarly the contents of all the second bins, and so forth. In each case, the sum was divided by the number of trials; the result was a fraction denoting the proportion of times that a unit detection had been made in the indicated time interval. Each of the first 100 intervals (which were prior to stimulus application) provided separate estimates of the proportions to be expected from a random selection of a number of time bins equal to the number of trials. The time bins immediately following stimulus application provided an estimate of the change in background firing rate caused by application of the auditory stimulus. Similar analyses were made of data derived from the movement detector. In this case, the "trigger level crossings" were substituted for unit detections. Movements occurred in response to the auditory stimulus at about 80-150 msec. after its onset. The analysis of the unit data was conducted in two ways. In one case, only the first 8 bins following stimulus onset were considered in order to eliminate unit effects fed back from behavior (called early-response analysis). In the second case, the entire second following stimulus onset was considered by quarter-second intervals (called the quarter-second analysis).

Responses and Latencies

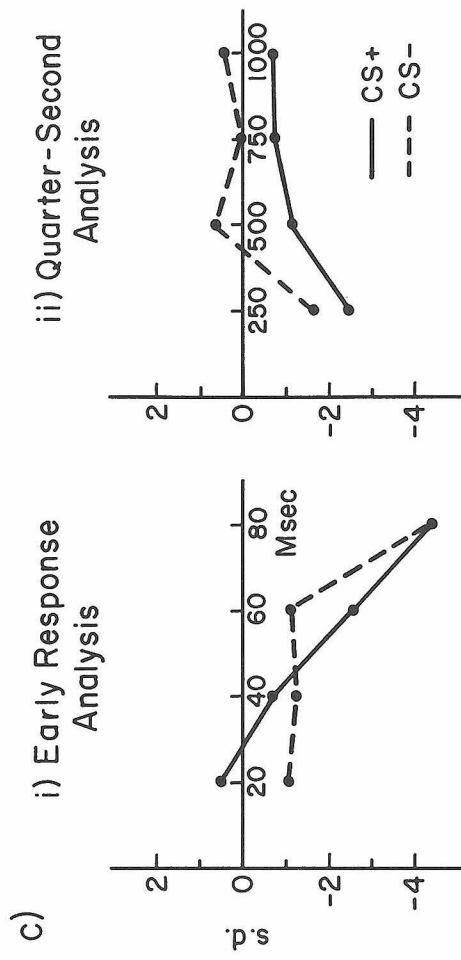
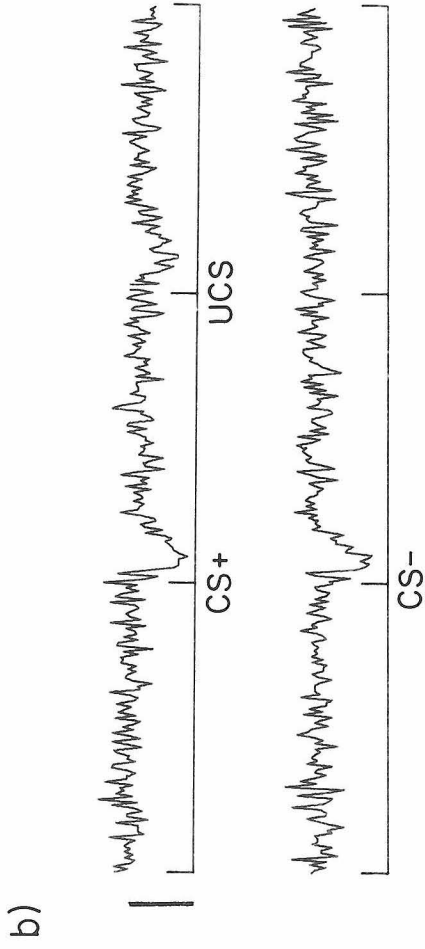
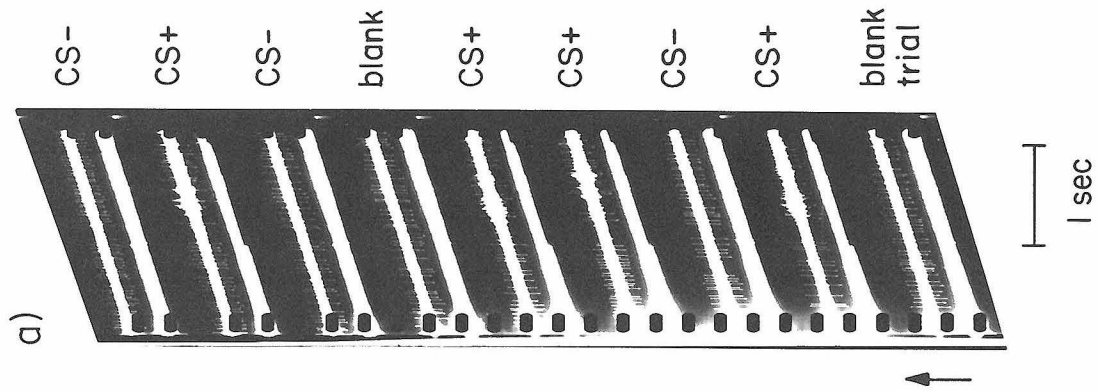
A "response" was an acceleration or deceleration of unit spike rate caused by presentation of the auditory signal. The method of analysis was to establish a mean and standard deviation on the basis of the first 100 (pre-stimulus) bins. In the early response analysis, the post-stimulus bins were then grouped in twos: 1 and 2 = the period from 0-20 msec. after stimulation; 3 and 4 = the period from 20-40 and so forth. The average rate in each group of trials (the two groups of 150 on pseudoconditioning day, and the 300 trials of conditioning day) for each of these pairs was computed separately and this rate was converted into a standardized deviation by subtracting the background mean over these trials and dividing by the standard deviation of the background mean. A response was considered to characterize a time interval (0-20, 20-40, 40-60, or 60-80 msec. after stimulus onset) if the average score for the pair of bins involved was at least 1.55 standard deviations ($p < .03$, two-tailed) from the mean background rate. The end of the first time interval to show such a deviation was counted as the latency of the response. Latencies were stated as 20, 40, 60 msec. and so forth from the onset of auditory stimulation. Actually, the tone had an air travel time of 4 msec. before reaching the rat's ear, so that the latencies 20, 40, 60 and 80 really represent maximally 16, 36, 56, and 76 msec. respectively.

The quarter-second analysis was conducted in a similar manner.

The post-stimulus second was divided into 1/4 second periods; bins 1-25 = the period from 0-250 msec. after stimulation; 26-50 = the period from 250-500 msec. and so forth. The average rate for each of these groups was computed separately and this rate was also converted into a standardized deviation. A response was considered to characterize a time interval if the average score for the group of bins was at least 0.5 standard deviations ($p < .001$, two-tailed, a smaller value than required of the 20 msec. interval because of the larger sample size from which it was drawn) from the mean background rate. These steps in data reduction are illustrated in Fig. 3. The latency of an unconditioned response was considered to be the end of the first time interval which showed a significant deviation in the same direction throughout the first day (pseudoconditioning) and the second day (conditioning). The latency of a conditioned response was counted as the end of the first time interval which after conditioning showed a significant deviation from the background rate and was also twice any deviation in the same direction on the second half of pseudoconditioning day. A comparison was made of the standard deviations of the 150-trial (pseudoconditioning) and 300-trial groups (conditioning) groups to check that differences did not depend on changes in variance. The difference was not significant (mean s.d. on Day 1, 0.013 (where the unit is the mean probability of firing per 10 msec. bin), mean s.d. on Day 2, 0.011; $t = 1.65$, $p = .10$, two-tailed).

Figure 3.

Steps in data reduction. a) Filmstrip showing activity of actual unit over a series of trials of the conditioning session (Day 2). b) Probability of firing histograms obtained by averaging the probabilities of response per 10 msec. period to the CS+ and CS- over approximately 300 trials of conditioning. The vertical bar to the left represents a probability of firing of 0.2 per 10 msec. period. c) Graphic representation of deviation scores obtained for CS+ and CS-, i) during the initial four 20-msec. periods, and ii) for the quarter-second intervals. According to the criteria used, the latency of this response to the CS+ (in this case, an inhibitory response) is 60 msec. In this case, as in many cases, there was also a conditioned response to the CS-. The latency of the response to the CS-, however, is 80 msec., and it terminates prior to the response to the CS+.



The criteria of conditioning were limited to identifying changes caused by conditioning if these consisted of the appearance of responses where there were none before, or of doubling of pre-existing responses (whether these were in the excitatory or inhibitory direction). The criteria were chosen to assure 1) that there was a marked change caused by conditioning, and 2) that the change did not represent merely the disappearance of a prior response. There were several instances in which previous responses apparently disappeared during conditioning, but since their interpretation was ambiguous - that is, it was not clear whether they reflected habituation that was complete only at the end of the first day, or were an important consequence of the conditioning procedure - they were temporarily set aside. The criteria described above were not considered to be statistical procedures so much as objective criteria for selection of new responses.

Learning Curves

An attempt was also made to examine the emergence of the learned responses over the sequence of the approximately 300 conditioning trials. Accordingly, for each probe showing a conditioned response and for which data were available for both the pseudoconditioning and conditioning days, the post-stimulus histogram on the conditioning day was inspected to determine the "response-interval". This consisted of that portion of the CS-UCS interval which showed a continuous

change in the same direction, outside the range of variability observed in the background activity of the pre-stimulus interval.

The data for both the pseudoconditioning and conditioning days were then reanalyzed to obtain "learning curves" which showed the changes in response size to the CS as a function of training trials. These learning curves were obtained by determining the average probability of firing of the unit per 10-msec. bin in the response-interval, and subtracting from this the average background probability of firing on the same trial. This difference in firing rate from background was plotted for both CS+ and CS- in 10-trial groups. The average pre-stimulus background rate for those 20 trials (10 each for CS+ and CS-) was also plotted. These provided curves for the first 270 trials of pseudoconditioning and for 20 pre-pairing trials and the first 250 trials following pairing during conditioning. The learning curves presented for the various brain regions and behavior are mean curves made up of the averages of the individual curves in the group (see Fig. 10). Each group curve was analyzed with a one-way analysis of variance to test for variation among the points on the curve. A significant result on this test indicated that there was a consistent increase or decrease within the curve. Analyses were also made of each curve on the conditioning day to determine the initial point of changed responsiveness to the CS+.

Very often, the responses of units studied were biphasic, that is, the unit responded to the CS by both an acceleration and a

deceleration of unit rate at different points during the CS-UCS interval. (For such an example, see Fig. 12d.) In many of these cases, two "response-intervals" were specified, one inhibitory and one excitatory. The excitatory responses only were included in the anatomical group curves. Units showing inhibitory responses, since they were in the minority, were represented as a separate group (see Fig. 13). This was done so that excitatory and inhibitory responses would not cancel one another in the averaged curves.

Generalization

In order to assess the amplitude of the generalized response, the response of a particular unit during the post-CS second was reduced to a single percentage score. This was done for both the CS+ and CS- trials by computing the average probability of firing per 10 msec. bin during the entire CS-UCS interval and expressing it as a percentage of the average probability during the background second. Again, since there were many biphasic responses, there was the possibility that inhibitory and excitatory responses would combine to show no change in probability of response to the CS. To avoid this, the value for probability was computed using the absolute value of the deviation scores. Thus, a response that showed a decreased probability of firing during the interval was represented as showing an increased probability of the same magnitude. Though

this was not strictly true, it was considered acceptable in order to be able to compute a percentage "change" score for these particular units. These percentage scores were then averaged for units within each anatomical area. The percentage scores to the CS+ and CS- were compared for each group to determine which areas showed differentiation between the two stimuli during conditioning.

RESULTS

Acceptable data were obtained from 118 probes in 80 animals. Units were simultaneously recorded from more than one probe in each animal. The conditioned responses observed, however, were usually different, and therefore were considered to be a function of the location of the probe and not of the animal (see Fig. 4).

Unconditioned Responses

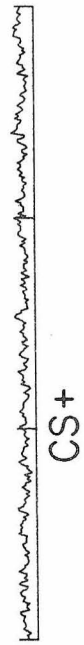
Unconditioned responses, defined as significant responses of constant latency, within an 80 msec. post-CS period, that remained throughout the experiment, were recorded from 24 of the 118 units or 20% of the cases (see Table 1 and Fig. 5 for their distribution and latency). In general, these were not specific to a particular tone; 17 out of 24 showed an unconditioned response to the CS- as well as to the CS+. The largest number of these unconditioned responses were found in the hypothalamus where they were characteristic of 10 out of 25 (40%) of the units. The hypothalamus also showed the largest proportion of unconditioned responses (4 out of 25) of the very shortest latency, less than 20 msec. after CS onset. Unconditioned responses were nearly absent in the pyriform-amygdala and preoptic areas where they were true of only one unit in each area.

Figure 4.

Probability of firing histograms showing three different conditioned unit responses for one animal, an early excitatory response in one hypothalamic unit, an early inhibitory response followed by a later excitatory response in the preoptic unit, and an unconditioned response, followed by a new conditioned response in the second hypothalamic unit. The vertical bars to the left of the traces represent a firing probability of .05 per 10 msec. period. In all cases these scales are the same for both pseudoconditioning and conditioning days.

PSEUDOCONDITIONING

Behavior



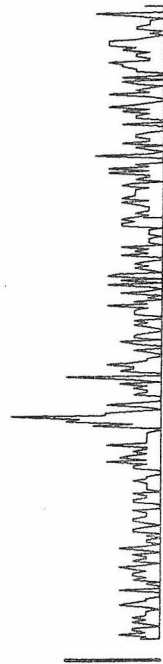
Hypothalamic Unit:



Preoptic Unit:



Hypothalamic Unit:



CONDITIONING

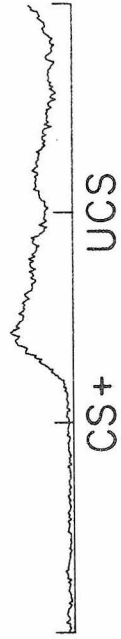


Table 1

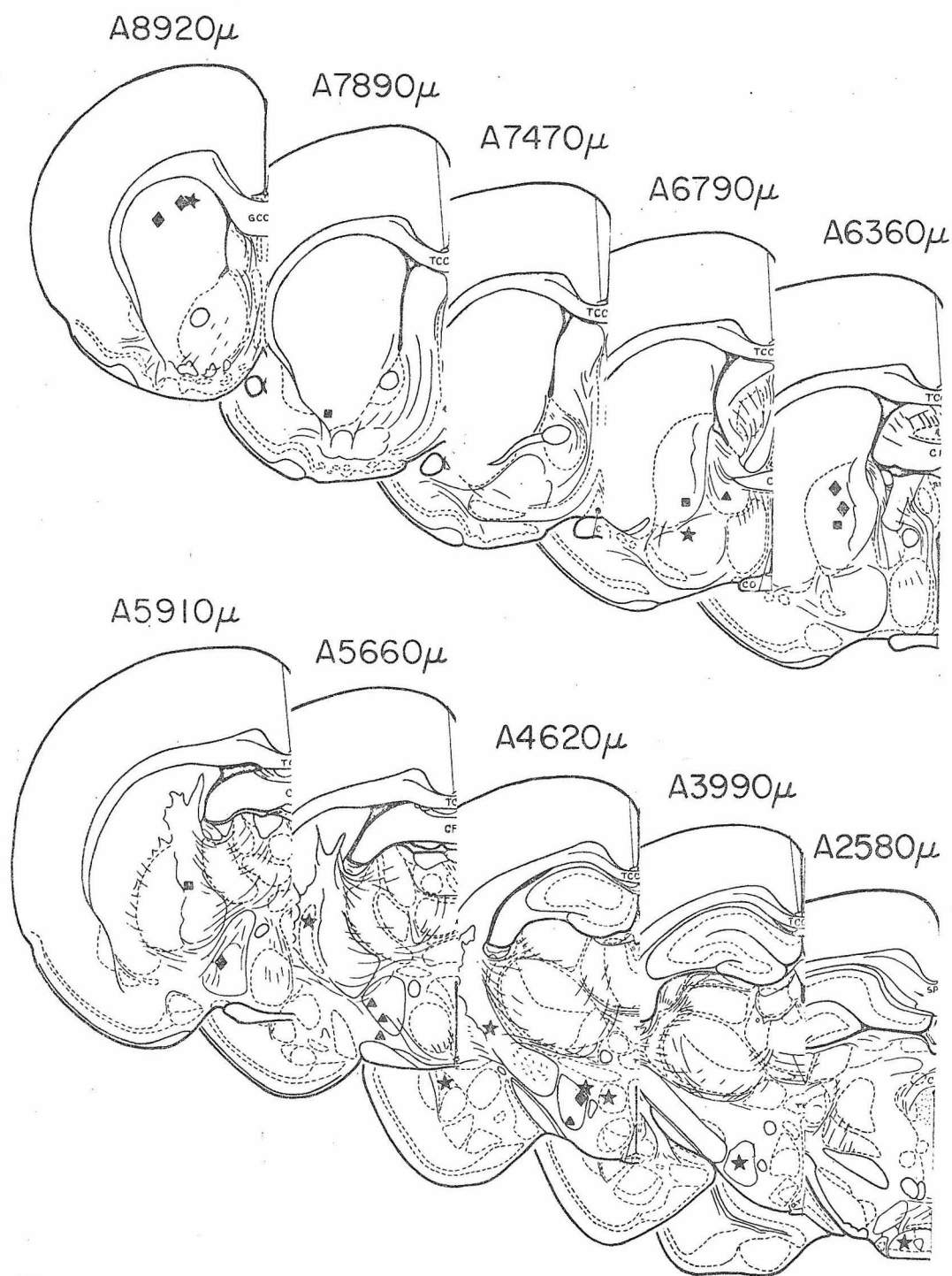
Unconditioned Responses

Area/Latency	20	40	60	80	Total
Hypothalamus	4	2	0	4	10
Preoptic Area	1	0	0	0	1
Internal Capsule	1	0	1	0	2
Globus Pallidus	1	2	2	0	5
Caudate Nucleus	1	2	1	0	4
Amygdala	1	0	0	0	1
Miscellaneous (Interstitial N. of Stria Terminalis)	0	0	0	1	1
					<hr/> 24

Figure 5.

Latency and distribution of unconditioned responses. Atlas sections are from Konig and Klippel (1963). For comparison with all points tested, see Fig. 6.

LATENCY AND DISTRIBUTION OF UNCONDITIONED RESPONSES



Conditioned Responses

A very large proportion of units showed significant conditioned responses within the one-second CS-UCS interval, 111 of the 118 units, or 94%; the majority of these had responses within the first quarter-second interval (see Table 2 and Fig. 6). Approximately half of these (52 cases) had conditioned responses within the first 80 msec. (prior to the behavioral response).

All but one of the units which had unconditioned responses showed an additional change as a result of conditioning. However, the change caused by conditioning was often in a later component, and thus the latency of the conditioned response was later than the latency of the unconditioned response. In only 4 cases was the earliest latency change an augmentation of a previous unconditioned response which characterized the same latency interval.

Very short latency conditioned responses (of less than 20 msec.) characterized 8 units, 3 in preoptic area and one each in hypothalamus, internal capsule, globus pallidus, caudate, and amygdala (see Fig. 7). Although this seems a great diversity of areas, all except one case (in hypothalamus) were close to one another in the ventrolateral forebrain area. The largest concentration was in the preoptic area and the others were near the borders of structures immediately adjacent to it. Two of the 8 cases might be ruled out by adding the requirement that the conditioned response must characterize not

Table 2

Latency of Conditioned Responses

a) Early Response Analysis

Latency Area	20	40	60	80	Total	% of Total Units Tested
Hypothalamus	1	6	5	2	14	56
Preoptic Area	3	3	2	2	10	50
Internal Capsule	1	2	2	1	6	43
Globus Pallidus	1	6	3	1	11	50
Caudate	1	0	4	0	5	26
Pyriform-Amygdala*	1	1	0	1	3	23
Miscellaneous**	0	1	2	0	3	60

b) Quarter-Second Analysis

Latency Area	250	500	750	1000	No Response	Total
Hypothalamus	18	4	1	0	2	25
Preoptic Area	16	3	1	0	0	20
Internal Capsule	7	3	1	2	1	14
Globus Pallidus	13	7	0	1	1	22
Caudate	8	7	1	2	1	19
Pyriform-Amygdala	7	1	2	1	2	13
Miscellaneous	5	0	0	0	0	5

Table 2 (continued)

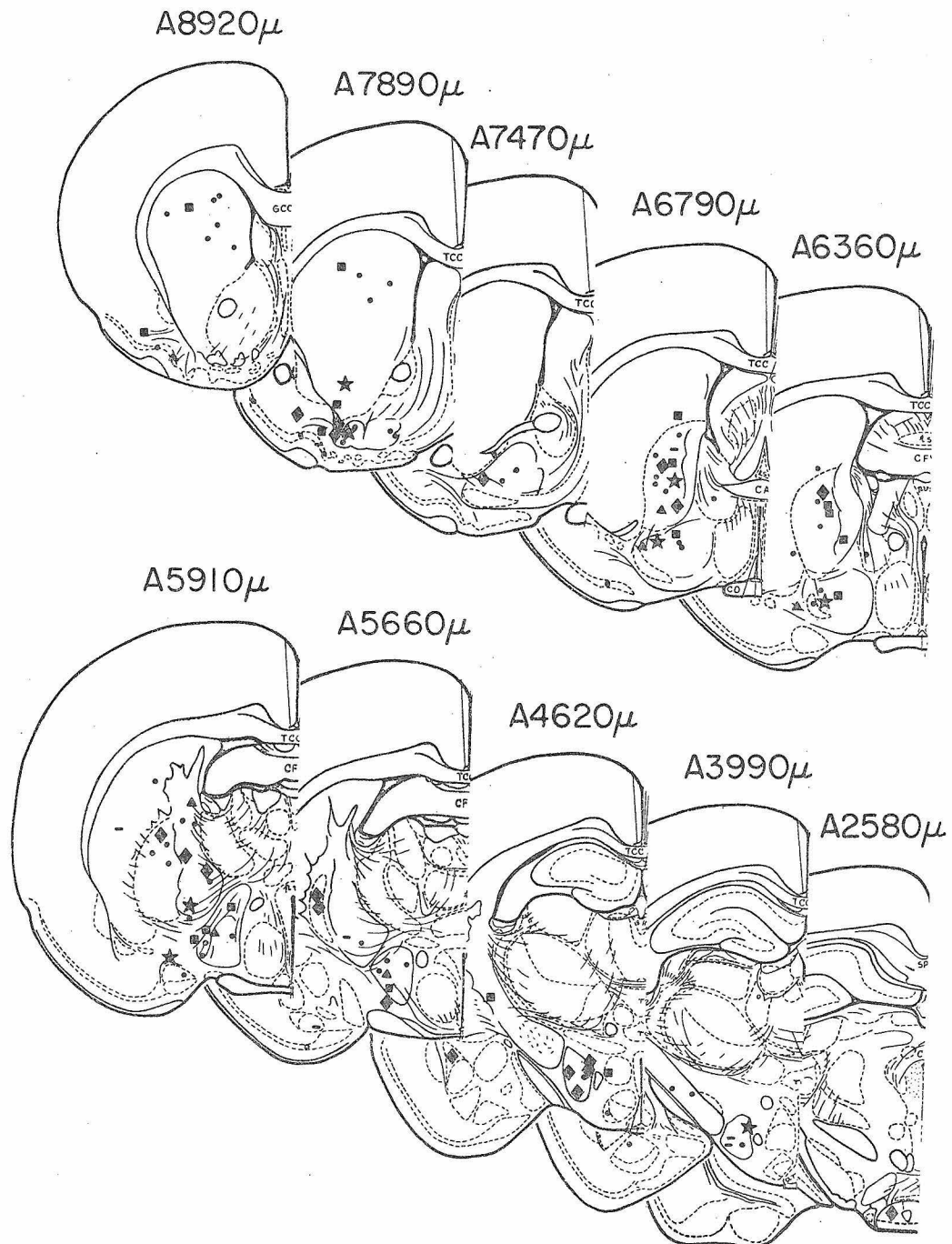
*Pyriform-Amygdala were grouped together because of their small numbers. The earliest responses were found in Amygdala.

**Miscellaneous includes Lateral Cortico-Hypothalamic Tract and Interstitial N. of Stria Terminalis.

Figure 6.

Latency and distribution of conditioned responses. Atlas sections are from Konig and Klippel (1963). For comparison with points showing unconditioned responses, see Fig. 5.

LATENCY OF CONDITIONED RESPONSES



Key:

- ★ 0-20 msec; ◆ 20-40 msec; ▪ 40-60 msec;
- ▲ 60-80 msec; • >80 msec; - No conditioned response.

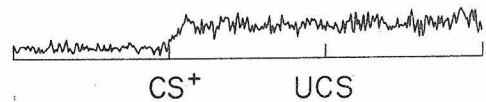
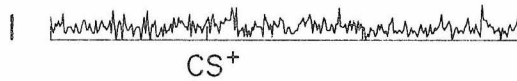
Figure 7.

Probability of firing histograms of all units, on pseudo-conditioning and conditioning days, showing very early latency (20 msec.) conditioned responses. The vertical bars to the left of the traces represent a firing probability of .10 per 10 msec. period.

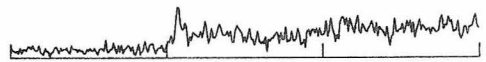
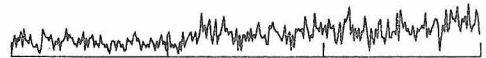
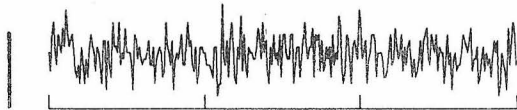
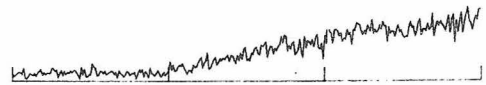
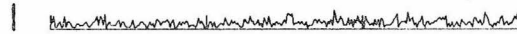
EARLY LATENCY CONDITIONED RESPONSES

PSEUDOCONDITIONING	CONDITIONING
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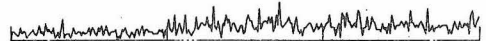
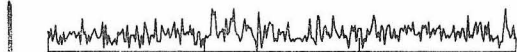
Hypothalamus



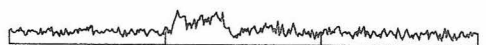
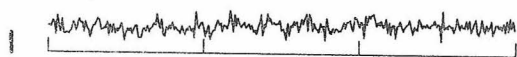
Preoptic Area



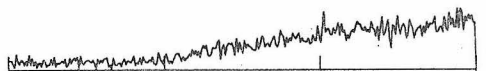
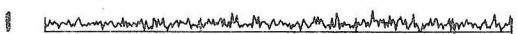
Internal Capsule



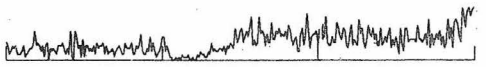
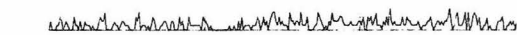
Globus Pallidus



Caudate Nucleus



Anterior Amygdala



only the 20 msec. latency interval but at least one other interval in the first 80 msec. period. The two cases removed by the more stringent criterion were in the caudate and preoptic groups. None of the very short latency CR's was generalized to the CS-. An additional search showed however that there were 6 cases where 20 msec. responses occurred to the CS- but not to the CS+, 3 in globus pallidus, 2 in internal capsule, and one in hypothalamus.

None of the conditioned responses of 20 msec. latency showed an unconditioned response of comparable latency. There was only one 20 msec. latency conditioned response in the hypothalamus, in part due to the large number of unconditioned responses of 20 msec. latency which characterized these units and their failure to be doubled by the conditioning procedure (and therefore to meet the criterion). This difference between most hypothalamic early responses and forebrain early responses is illustrated by the comparison between a hypothalamic and preoptic unit response in Fig. 8.

When the larger group of units (52 in all) with latencies up to 80 msec. were considered, these were more frequent in hypothalamus (56%), preoptic area (50%), globus pallidus (50%), and internal capsule (43%), than in the caudate nucleus (26%) and pyriform-amygdala (23%). The large proportion of caudate units failing to respond within this time interval (well before behavior) may be correlated with the fact that many caudate units appeared to fire only during overt movement of the animal (see Fig. 9 for comparison of a "phasic" unit which fired during movement with a spontaneously

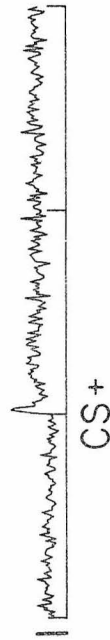
Figure 8.

Comparison of responses of a hypothalamic and preoptic unit. The hypothalamic unit shows a very early unconditioned response throughout the experiment which does not increase in amplitude with conditioning, but is followed by a new conditioned response. The preoptic unit shows no unconditioned response to the CS, but a very early new response as a result of conditioning. The vertical bars to the left of the traces represents a probability of firing of .10 per 10 msec. bin.

HYPOTHALAMIC UNIT

PREOPTIC AREA UNIT

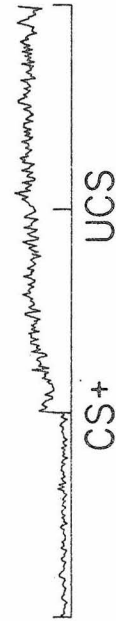
Pseudoconditioning — trials 1-150:



Pseudoconditioning — trials 151-300:



Conditioning:



UCS

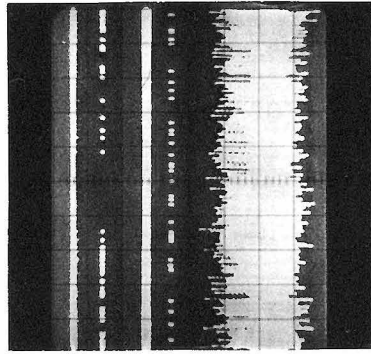
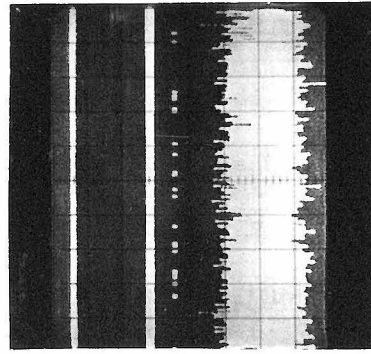


Figure 9.

Polaroid photographs of oscilloscope traces comparing "spontaneously active" and "phasic" caudate units, and their presence or absence during nonspecific movement of the animal.

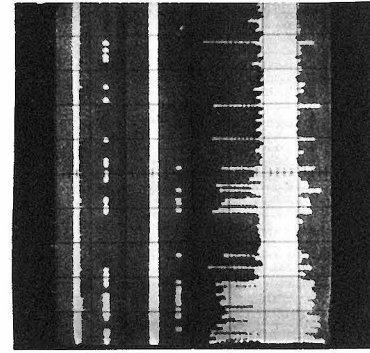
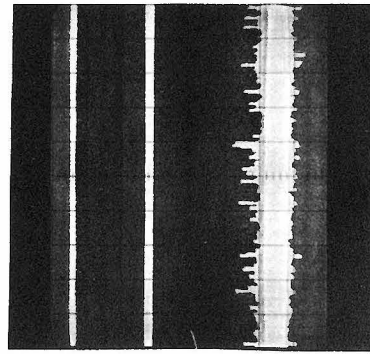
CAUDATE UNITS

a) SPONTANEOUSLY ACTIVE:



Movement detector output
Unit discriminator output
Unit activity

b) PHASIC:



2 seconds

active caudate unit).

The seven units (6%) that failed to show any change as a result of conditioning were not concentrated in any one part of the region studied.

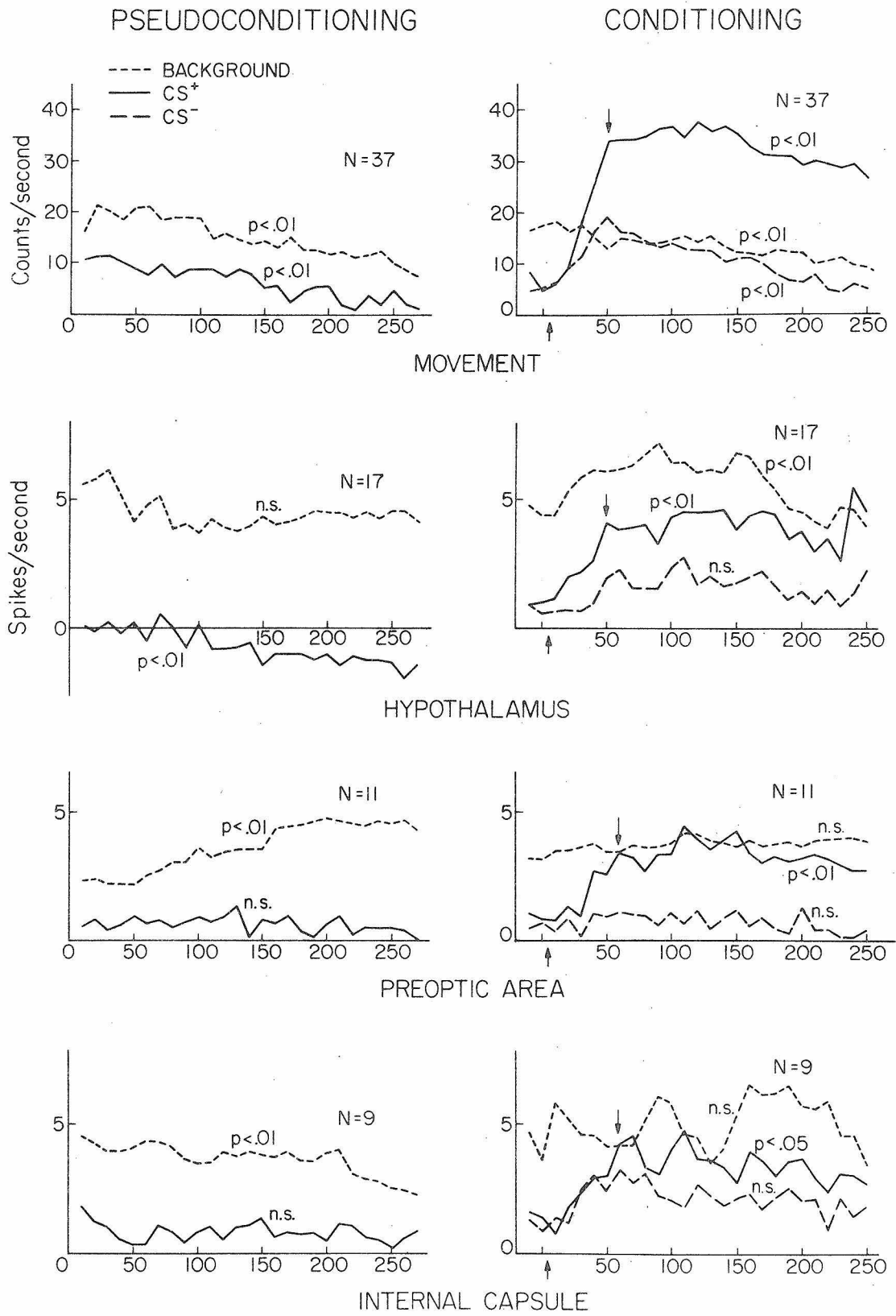
Learning Curves

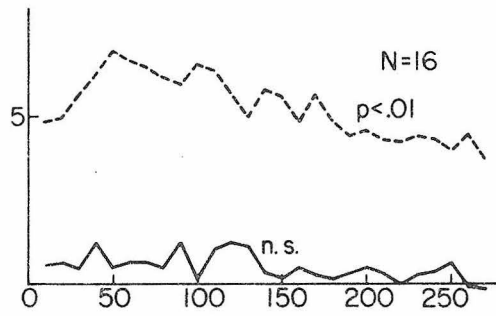
In all brain areas, except the pyriform-amygdala, there was an increase in response to the CS+ over trials on the conditioning day (one-way analysis of variance, $p < .01$, except the internal capsule where $p < .05$; see Fig. 10). Only in the caudate nucleus was there a similar change (i.e. significant by the same test) in responsiveness to the CS- ($p < .01$). The pyriform-amygdala group failed to show a significant trend in its response to the CS+, but in this group the n was small; changes in the average curve appeared but these were attributable to the response of one amygdaloid unit (see Fig. 10). On the pseudoconditioning day, only one area showed a changed response to the CS+; this was the hypothalamus, where there was a decrease in response over trials ($p < .01$).

There were several changes in background firing rates on each of the two days. On Day 1, there was a significant increase ($p < .01$) in the rate of preoptic units, and significant decreases in internal capsule, and globus pallidus ($p < .01$). The decrements were correlated with similar decremental changes in behavior ($p < .01$). On Day 2,

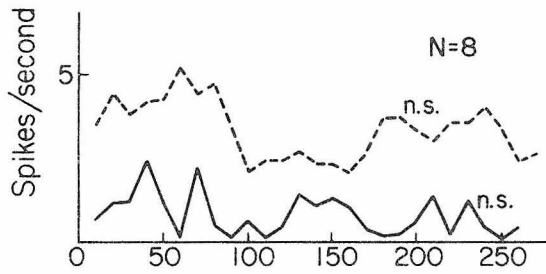
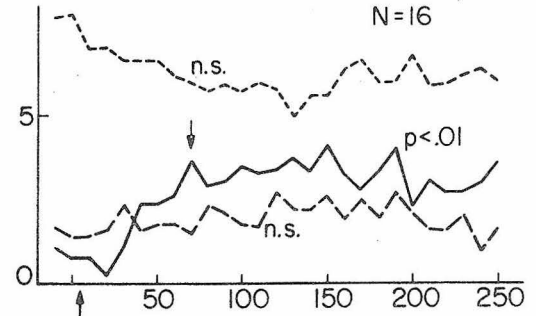
Figure 10.

Mean curves for behavior and units in each brain area studied during pseudoconditioning and conditioning. The background curves represent 20-trial periods for each unit and movement member of the respective groups, the CS+ and CS- curves represent 10-trial periods. The curves cover 270 trials during pseudoconditioning, 20 pseudoconditioning and 250 conditioning trials in succession on conditioning night. The CS+ and CS- curves are expressed as response minus background. The significance level appended to each curve is the result of a one-way analysis of variance with repeated measures done for each curve. The arrows along the abscissa of the conditioning day curves indicate the point at which pairing began. The arrows along the CS+ curve on conditioning day indicate the first point to emerge significantly from background according to Dunnett's procedure ($p < .05$, Winer, 1962).

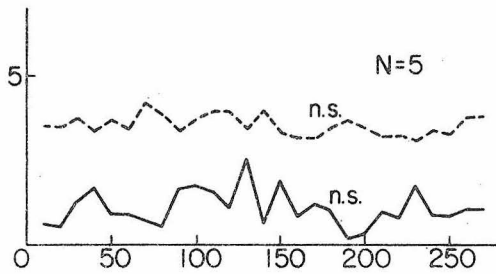
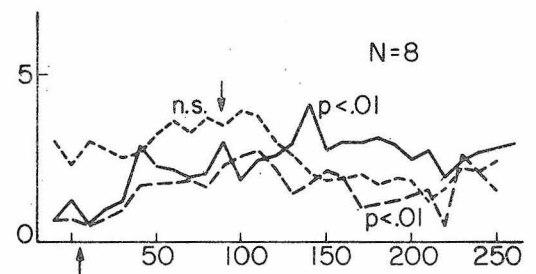




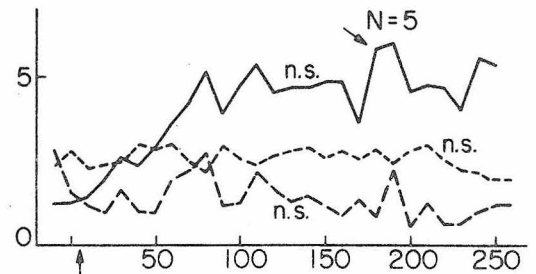
GLOBUS PALLIDUS



CAUDATE NUCLEUS



PYRIFORM-AMYGDALA



there was a further decrease in the behavior scores ($p < .01$). At the same time there was a complex change, an increase followed by a decrease in the background firing rates of hypothalamic units ($p < .01$). This was particularly interesting because the onset of the complex series of changes was very closely correlated in time with the commencement of the conditioning procedure.

In order to permit comparison of different areas with respect to the time of emergence of conditioned unit responses on Day 2, the first point along each curve to differ significantly from the background was determined (0.05 level, Dunnett's procedure; Winer, 1962). The time of emergence of the behavioral CR was determined from the movement detection data by the same test. The behavioral CR according to this test emerged during the third group of 10 trials following the start of conditioning. The unit CR's in the brain emerged at the fifth group of ten trials for the hypothalamus, the sixth group for the preoptic area and the internal capsule, the seventh group for the globus pallidus, the ninth group for the caudate nucleus, and the eighteenth group for the pyriform-amygdala units.

Because this method did not allow for a test of the differences between groups, a second procedure was also adopted. This was to determine for each unit the number of 10-trial groups required to reach a selected criterion (namely three successive 10-trial averages

above the last two preconditioning 10-trial averages). The first of the 3 was taken as indicating the 10 trials during which learning had occurred (see Table 3). Within the hypothalamic-forebrain groups of units there were no significant differences by this test. The whole group of units, however, learned significantly earlier than cortical units which had been tested the same way ($p < .01$; Disterhoft and Olds, in press).

Gross inspection of the learning curves appeared to show that substantial changes occurred in hypothalamus before behavioral learning, and that extrapyramidal units showed signs of change somewhat later than the first signs of behavioral learning. The small number of cases, however, does not permit statistical substantiation of this observation.

Inhibitory Responses

One feature of particular interest was the number of inhibitory conditioned responses. The units in these cases responded to the CS+ by a decrease in rate of firing. Such responses characterized 23 out of the 118 units tested, or 20% (see Fig. 11). This was a considerably larger proportion than found in other parts of the brain studied by similar methods (Segal and Olds, in press; Disterhoft and Olds, in press; Kornblith and Olds, submitted). Although they were found in all structures studied in this experiment, they were considerably more frequent in three areas, comprising 35% of the

Table 3. Initial Point of Learning Analysis

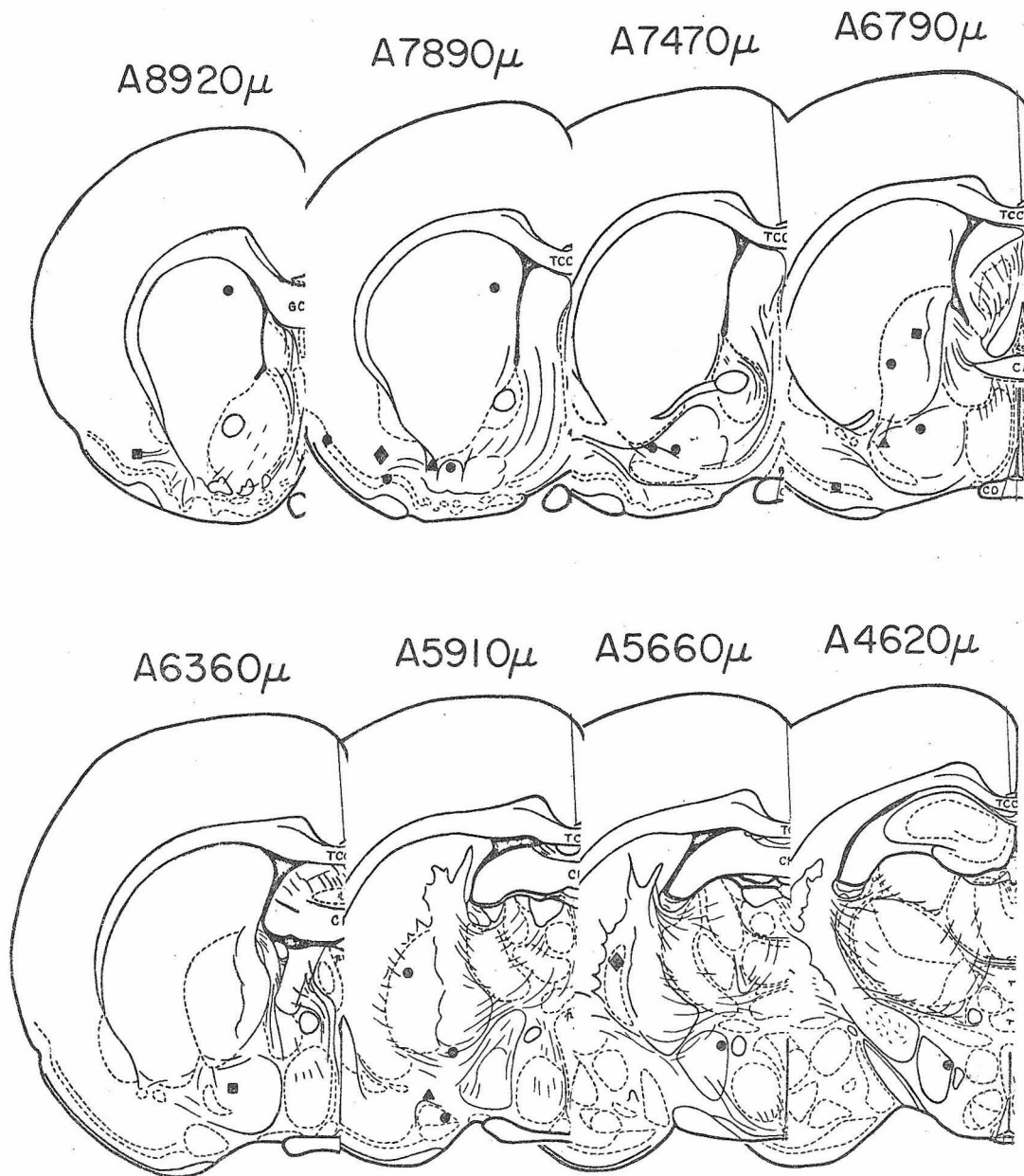
	Mean No. of 10 trial groups	S.d.	N
Hypothalamus	5.63	6.83	16
Preoptic Area	3.40	2.63	10
Internal Capsule	4.43	2.37	7
Globus Pallidus	4.64	3.32	14
Caudate	3.00	2.00	5
Pyriform-Amygdala	8.20	7.89	5
Total	4.88	3.74	57
Movement Behavior	3.41	3.06	34
Cortex*	6.87	3.14	66
Pyriform-Amygdala >< Behavior	t = 2.45, p < .02		
Cortex >< Total Subcortical Group	t = 4.98, p < .01		
Total Subcortical Group >< Behavior	t = 3.50, p < .01		
Cortex >< Behavior	t = 8.85, p < .01		

*From Disterhoft and Olds, in press.

Figure 11.

Latency and distribution of inhibitory responses. For comparison with all points tested, see Fig. 6. This figure does not include the two most posterior sections illustrated previously, as there were no inhibitory responses in those posterior regions of hypothalamus. Atlas sections are from Konig and Klippel (1963).

LATENCY AND DISTRIBUTION OF INHIBITORY RESPONSES



Key:

- \blacklozenge 20-40 msec; \blacksquare 40-60 msec; \blacktriangle 60-80 msec;
- \bullet >80 msec

responses in the preoptic area, 39% in pyriform-amygdaloid areas (3 in pyriform, and 2 in anterior and corticomедial amygdala), and 2 out of 3 units in the lateral cortico-hypothalamic tract. There were fewer in globus pallidus (18%), caudate (11%), hypothalamus (8%) and internal capsule (7%). In general, these inhibitory responses did not have early latencies - the earliest occurring at 40 msec. post-CS, but the majority much later. Most of these responses were transient, lasting often less than a quarter of a second, and terminating well before the end of the interstimulus interval. There were some exceptions, particularly of note, two hypothalamic responses, which occurred later in the interval and extended in time past the feeder presentation.

Although the inhibitory response was the only significant response of some units, most often it was part of a biphasic or multiphasic response. That is, it was preceded by an excitatory response, or more frequently followed by an excitatory response. In some instances, there was a combination of these (see Fig. 12 for some examples).

There was only one case of an inhibitory unconditioned response, in the hypothalamus, but this unit did not show a learned response of this kind on the conditioning day.

A separate "learning curve" was computed for the group of units showing inhibitory conditioned responses. However, in this case, there was no consistent significant change in responsiveness to the CS+ on conditioning day, as might have been expected (see Fig. 13).

Figure 12.

Forms of inhibitory responses. Responses of units showing:

a) only an early inhibitory response, b) a very early unconditioned response followed by the inhibitory response, c) an early transient excitatory response followed by a short inhibitory response, d) an early inhibitory response followed by late excitatory conditioned response, e) an early excitatory conditioned response followed by transient inhibitory response, and followed by a later excitatory conditioned response, and f) hypothalamic unit response, with an early unconditioned response, an early excitatory conditioned response, and a later sustained inhibitory response. The vertical bars to the left of the traces represent a firing probability of .10 per 10 msec. period.

INHIBITORY RESPONSES

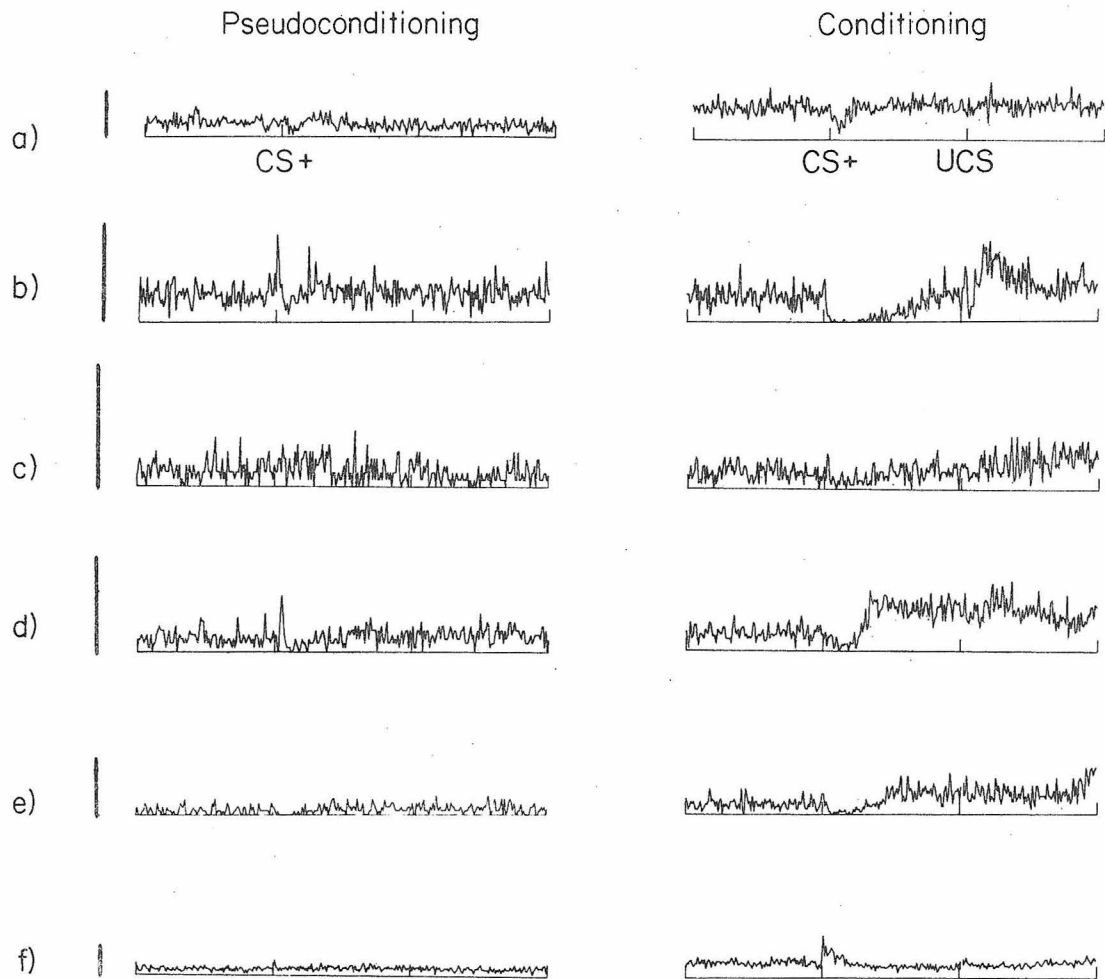
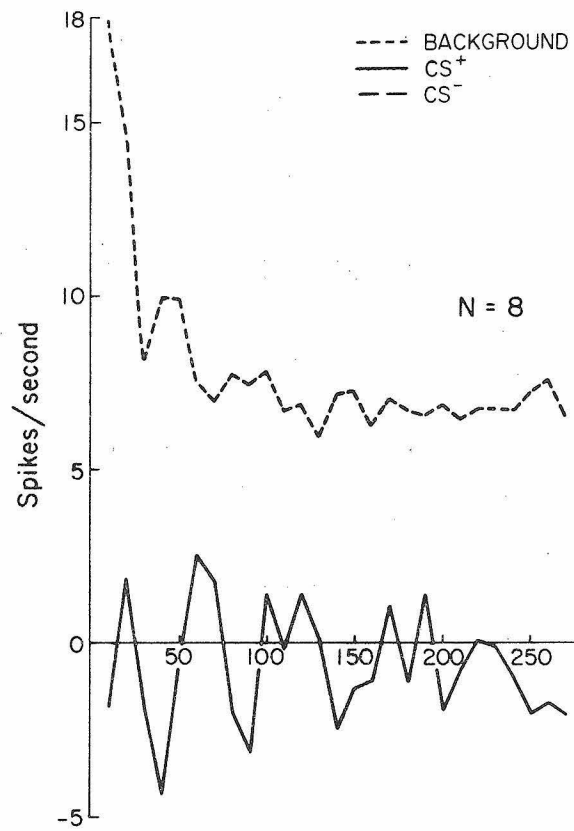


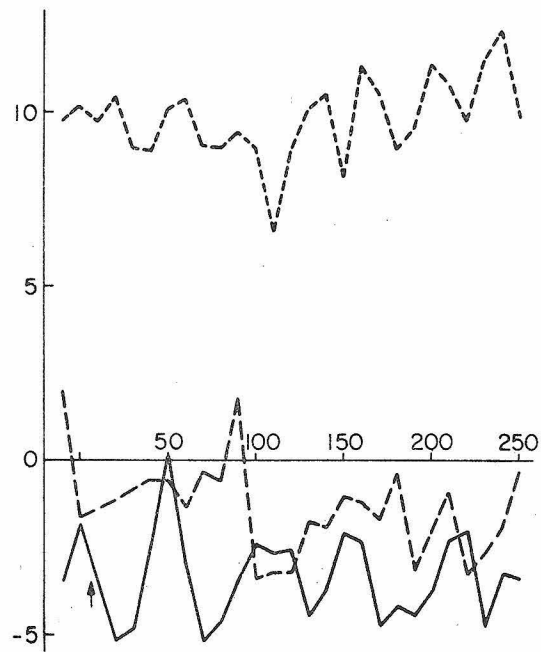
Figure 13.

Mean curves over trials for units showing inhibitory responses, during pseudoconditioning and conditioning. The background curves are derived as before. The CS+ and CS- curves represent only that interval over trials in which the inhibitory response was present, as judged from the average histograms. There was no significant trend in any curve as determined by a one-way analysis of variance of repeated measures over trials.

PSEUDOCONDITIONING



CONDITIONING



These inhibitory responses did not yield good "learning curves" because there was often no continuous change in the unit responses (nor even any trend) from the beginning to the end of the learning day. Nevertheless, the average responses on the conditioning day were often double the responses in the same direction on the day before, and thus the criterion for conditioning was met. The lack of a trend was mainly due to the fact that the inhibitory responses were large at the beginning of both days (when the stimulus was novel or when its novelty was renewed by time). On the pseudoconditioning day, the large initial inhibitory response waxed and waned but averaged substantially above its starting level. On the conditioning day it also waxed and waned but averaged much closer to its starting level. In other words, it seems that if the curves were flattened, there was an inhibitory response which habituated on the first day and one that did not habituate on the second day.

Generalization

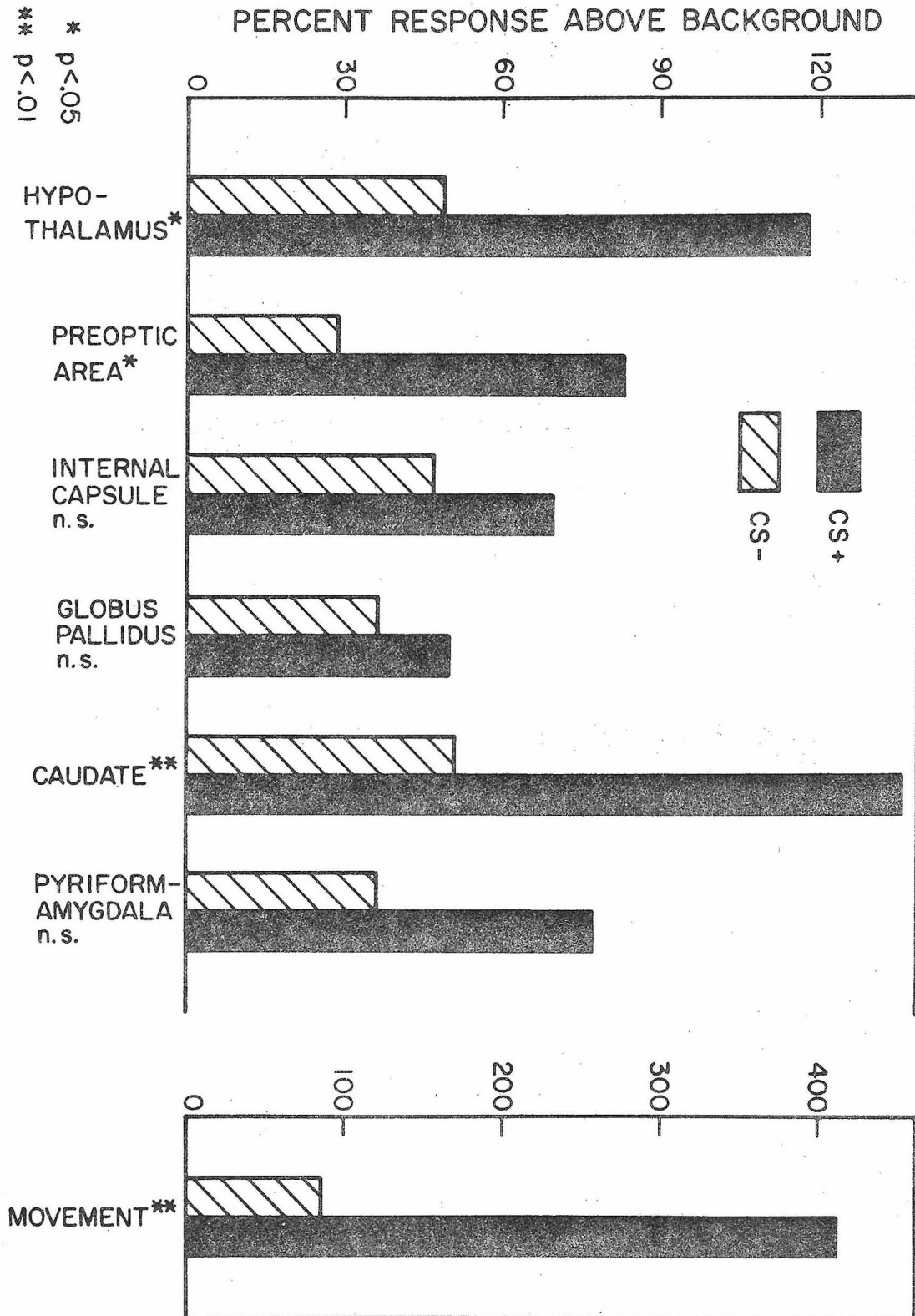
A comparison was made between the mean percent responses above background to the CS+ and CS- within each of the major areas on conditioning day (see Fig. 14). This comparison revealed there was a significant differentiation in response to the two conditioning stimuli among units of the hypothalamus, preoptic area (t-test, $p < .05$) and caudate, and in the behavioral response of the animal ($p < .01$). However, there was not a significant difference

among units of the internal capsule, globus pallidus and pyriform-amygdala areas.

An effort was also made to assess the degree of generalization of the inhibitory responses. Since the inhibitory response covered only a portion of the interval, it was necessary to compare the responses of only that part of the interval, in contrast with the entire one-second CS-UCS interval used previously. This was done by considering the deviation scores of the quarter-second interval in which the largest inhibitory response to the CS+ occurred. When the interval showing the largest response to the CS+ was compared to the same interval of response to the CS-, there was a significant differentiation of response (mean deviation score to CS+, -1.14; to CS-, -0.55; $p < .01$). When the peak intervals of response to the CS+ and CS- were compared (not necessarily the same quarter-second interval) the difference approached significance (mean deviation score to CS+, -1.14; to CS-, -0.74; $p < .08$).

Figure 14.

A comparison of mean percent response above background level to the CS+ and CS- for units of each major area studied, and for behavior. There was significant differentiation of response to the two stimuli among hypothalamic, preoptic area, and caudate units, and in behavior. Units of internal capsule, globus pallidus, and pyriform-amygdaloid areas did not show significant differentiation.



DISCUSSION

The Clustering of Short Latency Responses

Although, as in preceding recording studies, the number of units showing changes following conditioning was very large, only a small number of these (8 cases or 7%) showed very short latency responses, that is, of less than 20 msec. following CS onset. It is at these sites that local processes related to learning seem likely. These sites did not fall predominantly within any previously delineated anatomical structure. However, with the exception of one hypothalamic site, they all fell close together. This was in a ventrolateral forebrain area, close to the intersection of striatal and hypothalamic structures.

In addition to meeting the short latency criterion, these sites were considered likely sites of rerouting on neuroanatomical grounds. This is because there were a large number of hypothalamic units with short latency (probably sensory) unconditioned responses, and cells of the hypothalamus project forward, sometimes monosynaptically, into the regions of the ventral forebrain where the early latency conditioned responses were observed (Crosby, Humphrey and Lauer, 1962). This may mean that synapses not previously transmitting information beyond the hypothalamus during pseudoconditioning were changed so that they did successfully project the information to forebrain cells as a result of conditioning. The possibility of hypothalamic unconditioned responses driving basal forebrain

conditioned responses received some support from the rather clear separation of UCR's and CR's (see Figs. 5 and 6). That is, none of the units showing such early conditioned responses had shown unconditioned responses of comparable early latency.

The Problem of Day 1 Habituation

In areas where there was marked habituation on Day 1, it is plausible to argue that the post-habituation scores form the most appropriate basis for evaluation of the pre-conditioning responses. Therefore these were used and conditioned responses were defined as those cases where the rates were double those during this base period. However, counterarguments are possible. One would be that reinstatement of a previously habituated response would not qualify as conditioning. This is a matter of definition and does not seem to warrant further consideration. A more important argument would be that dishabituation might occur overnight; this would make Day 2 scores higher than those for the second half of Day 1 and would provide false evidence of an increase caused by conditioning. This argument does not get much support from the learning curves (that is, the data for the full CS-UCS interval). Only the hypothalamic group of scores showed a substantial overnight change in the "dishabituating direction", and these units had not shown any substantial response to the CS at the beginning of Day 1. The short latency UCR's which appeared in several cases were evidently too small, too brief, and

too rare to show in the group data based on the whole interval.

Because the learning curves that form the basis for the previous argument were composed of data for the whole CS-UCS interval, they still leave the question unanswered for the more important "20 msec." CR's. That these were probably not due to that kind of artifact is indicated by the fact that 5 of the 8 "20 msec." CR's were more than double even the first-half Day 1 scores. Three cases, one each in caudate, globus pallidus, and hypothalamus, would lose their "20 msec." CR status by this more stringent test.

The comparison in the hypothalamus of the first and second day "habituation and learning curves", for the whole CS-UCS interval, offers an interesting problem that merits further discussion. On Day 1, this group of units taken as a whole was at first unaffected by the CS, but as the behavioral response habituated, a suppression of hypothalamic units by the CS appeared. This results in an "inhibitory" response at the end of the first day. It was surprising therefore to find that at the beginning of the second day this inhibitory response was absent, and there was perhaps even a small excitatory response. It was as if an active process inhibiting the hypothalamic response developed as the animal habituated to the stimulus on the first day. But on the second day, when a now familiar stimulus was re-presented, it aroused more interest than it did initially, and hypothalamic neurons were consequently excited. It is as if the same "set" that developed to inhibit the responses on Day 1 had a part in exciting them at the beginning of Day 2.

The Clustering of Inhibitory Responses

A large number of inhibitory conditioned responses were recorded from the brain regions included in this study. It was a much larger proportion than appeared in any other brain areas studied in a similar manner (Olds et al., 1972). There appear to be two possible functional explanations of these responses. Forebrain inhibitory responses have been described and postulated to be characteristic of a food-reward behavior sequence (Travis, Hooten and Sparks, 1968; Berger, Wise and Stein, 1971). On the other hand, stimulation of these forebrain areas has been shown to result in cortical spindling and behavioral inhibition (Clemente and Serman, 1963; Buchwald, Wyers, Okuma and Heuser, 1961). Inhibition of cell activity in this region might therefore be correlated plausibly with a transient arousal. Experiment II was designed to test between these alternatives by comparing the responses when appetitive and aversive unconditioned stimuli were used. This problem will therefore be discussed in more detail later.

The Learning Curves

The points of emergence of new conditioned responses by areas were examined in two ways. In the analysis of the "learning curves" themselves, points emerged significantly from background earliest in hypothalamus and subsequently and almost successively in areas anterior to this, preoptic area and internal capsule, followed by

globus pallidus, then caudate, and pyriform-amygdala. This finding is comparable to another reported by Halas, Beardsley and Sandlie (1970). In a study of multiple unit activity during conditioning, they too found new responses appeared first in posterior structures of the brain (medullary reticular formation) and successively in more anterior areas, appearing last in cortex.

However, in this experiment, when the mean number of trials to a select criterion for each group were compared, no significant differences between groups were found. This may have been because of the small sample in some cases. When all these units were considered together as a subcortical group, and compared to a sample of cortical units studied similarly, a significant difference did emerge between groups, with the subcortical units achieving the criterion within fewer conditioning trials. Disterhoft and Olds (in press) previously found a significant difference between thalamic and cortical units, with conditioned responses in thalamus preceding those of cortex.

Such a pattern would appear to contradict a notion held earlier by many learning theorists regarding acquisition of new responses by the brain. That is, it was believed that new learning first took place in the cortex, on the assumption that an animal was most "conscious" of those responses most recently acquired. Then gradually, as the responses became more automatic with additional training, and thus required less conscious attention, their control was assumed by subcortical structures (Lashley, 1960). These data suggest that

the reverse is true, insofar as the rerouting of the stimulus message is concerned. Changes of response in the cortex occurred only after the new behavior was performed repeatedly over a period of time. These results thus support the theory advanced by Marr (1970) that the cortex stores memories of events that have occurred with some redundancy, or longer term memories. There is, however, the possibility that dynamic changes in cortex could precede and account for subcortical rerouting though new cortical responses to the CS itself appear much later.

Some caution should be exercised regarding the generality of these findings. Because of the extensive habituation and pseudo-conditioning procedure followed on Day 1, it may be that a whole set of processes re-establishing interest in the CS needed to precede conditioning on Day 2. This could possibly account for the priority of subcortical areas. Normally, when such prior "unlearning" is not required, all brain areas might learn at once.

Generalization

The amount of generalization of the conditioned responses could be judged in two ways. Comparing the percentage scores of responses to CS+ and CS- (as shown in Fig. 14) revealed that there was not a significant differentiation of responses to the two stimuli by unit groups of the globus pallidus, internal capsule, and pyriform-amygdaloid area. Hypothalamic, preoptic and caudate units did show significant

differences in response to the two stimuli. However, looking at the "learning curves" (as in Fig. 10), the caudate was the only region to show a significant change in responsiveness to the CS- as well as to the CS+.

The interesting point in these results is that those areas showing the most stimulus generalization, the internal capsule, globus pallidus, and caudate, are those areas - the basal ganglia - generally believed to be most involved in motor function. This is somewhat of a paradox. That is, this author had believed, a priori, that responses would tend to become more specific as a signal would proceed from the input (sensory) to output (motor) areas within the brain. The same paradox characterized motor as compared to sensory cortex (Disterhoft and Olds, in press). One might argue that there is as much activity of upper motor systems required to inhibit behavior when closely related stimuli are applied as to program it when the CS+ is applied. The inhibitory effects of caudate stimulation (Buchwald, Wyers, Lauprecht and Heuser, 1961) would be compatible with such a view.

The Short-Latency Responses to the CS-

On first thought, these six cases suggested the possibility that many of the short latency conditioned responses occurred by chance. However, the location of the short latency responses to the CS⁺ predominantly within the preoptic area where there was little

generalization, and those to the CS- within the basal ganglia where there was much generalization, seemed also to indicate a possible special involvement of the basal ganglia in the inhibition of response to the CS-.

Dynamic Engrams

Short latency conditioned responses have been interpreted as reflecting sites of local change due to learning. It may be objected that such responses might not, in fact, reflect local changes, but could result from the action of a dynamic trace (Horridge, 1968) residing elsewhere, whose effect would be projected to the site of the recording probe. That is, the training might cause an increase or decrease in the tonic firing rates of some neurons, which would then project upon a second set of synapses, and by its contribution effectively change the probability of transmission across these. A new conditioned response might then be recorded from the post-synaptic element, but the actual change would be in the firing rate of the tonically active cell. Such a change should be detectable as a change in the background activity of these "dynamic trace" cells. A significant increase in background firing rate following pairing was recorded in hypothalamus. This background change might possibly reflect a change in arousal caused by the sudden change in its stimulus environment. It could, however, be part of a temporary

representation of the new association. Because this increase in hypothalamic tonic firing level began to wane following about 150 trials, before the end of the experimental session, the idea of its participation in a temporary engram was attractive.

Latency Mapping

This study combined latency with mapping strategies, previously employed separately by Woody (1970) and John and Killam (1959), respectively, in order to localize possible sites of rerouting in the mammalian brain. Data gathered to date suggest that the temporal distinctions used here should be further refined, a course presently being pursued. That is, the bin width might be reduced in order to be able to measure latencies of response of less than 20 msec., and preferably to such a value that one would be able to estimate quite accurately the entire sequence of neuronal events between stimulus and response. This will reduce the number of supposed rerouting sites by discovering sequences among the 20 msec. latency responses, and will permit the argument that some of them result from projections from others. Furthermore, a much larger group of points is required to make any of the findings more than suggestive.

Further Research

There are two other interesting directions. One of these is to explore in detail the possibility of dynamic traces. This could be

done by mapping the origins of projections to the rerouting points in order to find whether dynamic engrams would be evidenced at these. The second is to study the so-called consolidation process. This would be done by examining the effect of additional trials or time on the long term characteristics of the conditioned response. That is, there may be changes which occur early, but then disappear when learning is complete, which could be determined by studies during overtraining. Some changes may disappear as a function of time alone, that is, be "forgotten"; for these "waiting" studies would be required. Other changes may appear or be augmented as a function of time alone or "consolidate"; these could also be detected by waiting studies.

Responses disappearing in overtraining or with time might be related to short-term memories, those being "consolidated" in time, to long-term memories. These different types of memories might be localized within different regions of the brain, and be to some degree independent of one another. The idea of independence of short and long term memories has been advanced by others (Kesner and Conner, 1972) who found that post-trial stimulation to reticular formation and hippocampus resulted in amnesias with different temporal characteristics. Stimulation of reticular formation resulted in poor recall on a retest after a one-minute delay, but had no effect on retention 24 hours later. Hippocampal stimulation had the opposite effect. It would be interesting if correlates of these effects could be found through recording.

It is assumed that the shortest latency points indicate local rerouting, though the nature of the change to account for it cannot yet be specified. Studies to date suggest at least two types of changes within the mammalian brain. These include structural changes, generally of synaptic growth (Valverde, 1967; Cragg, 1969; Raisman, 1969) as evidenced by electron microscopic studies some time following the learning experience; and biochemical changes, related to protein synthesis (Barondes, 1970; Hyden and Egyhazi, 1962) or transmitter availability (Rosenweig, 1962) found more immediately following the learning. This evidence, however, is derived from larger samples of brain in which learning has been inferred and not in any particular cells for which there is independent evidence of learning.

There are several drawbacks in the use of the present technique for further analytical purposes. It would be preferable to be able to record intracellularly as well as extracellularly, but this is possible for only certain classes of cells in the mammalian brain at present. One could thereby monitor the resting potential of the cell and any changes in threshold which may result as learning progresses. In addition, it would be preferable to be able to record repeatedly from identical cells, and to be able to do cellular biochemistry. It is conceivable, however, that certain analyses (e.g. electron microscopy, regional biochemistry) could be done upon certain samples of cells, for example, from an area showing a large

proportion of early new responses compared to cells in an area having shown none at all (e.g. posterior thalamus vs. dorsal caudate).

One attraction is toward a simpler model which lends itself to these additional analytical techniques. Such models are offered by several invertebrate systems currently being studied. However, the behavior of these animals is qualitatively different from that of mammals in most instances, and one might imagine that learning mechanisms have changed with evolution, if not in complexity, possibly in number. Hence, it will be difficult to generalize solutions discovered at the invertebrate level to the mammalian brain unless there is concomitant study at the vertebrate level. Even at the invertebrate level, two of the main problems would still exist. The changes of learning might not be detectable upon analyses of the cell body, but instead be local to particular ones among the many synaptic sites characteristic of a single cell. Furthermore, the neuropil might still be so complex as to make identification of synapses between particular cells difficult if not impossible. The single identifiable cell might have a seemingly insurmountable complexity of its own. There remains the possibility of cell culture in which individual synapses could be isolated but in which behavior learning would be all but impossible to demonstrate.

This points to the difficulty of the problem, the limitations of different systems in the study of learning, and the consequent value of several simultaneous and diverse approaches. It is to be hoped, however, that cell culture may indeed describe factors in

membrane attraction and synapse formation; that invertebrate models and learning analogs may reveal possible cellular mechanisms; and that these may be transposed directly or, at least in part, to more complex systems.

CONCLUSION

These data then suggest that there are important events related to learning occurring within the hypothalamic-striatal area. A small number of very early latency learned responses were recorded from sites mostly in the ventral forebrain area, close to the intersection of hypothalamic and striatal structures, indicating these may be points of rerouting of stimulus information following learning. In addition, the hypothalamus showed an interesting increase in background firing rate shortly after the correlation of the CS and UCS, suggestive of the possibility that it may represent a kind of dynamic trace of the new learning. This is in marked contrast to general notions regarding the functions of these areas - that is, that they are involved in homeostatic regulatory functions, fixed action pattern sequences and stereotyped motor movements, with little thought to a possible role in learning. Examination of the development of these new responses in the trial-to-trial sequence of learning, revealed that they appeared prior to new learned responses in the cortex, but possibly subsequent to new responses in the ventral tegmentum. Units of the basal ganglia showed larger generalized responses to the CS- than units of the hypothalamus or preoptic area, suggesting these areas may be importantly involved in the inhibition of response to the CS-. In addition, a large percentage of inhibitory conditioned

responses were recorded mostly in the ventral forebrain. Often these were preceded or succeeded by additional excitatory responses. An additional experiment was performed to determine whether these were more likely related to a specific feeding or general arousal function.

EXPERIMENT 2

INTRODUCTION

Experiment 1 revealed a number of inhibitory conditioned responses, concentrated in ventro-lateral regions of the forebrain. At present, it appears there are two possible interpretations regarding the function of these responses.

The first of these is that these inhibitory responses are related to the arousal of the animal. Lesions of the basal forebrain area have been found to cause sleeplessness in rats and cats (Nauta, 1946; McGinty and Serman, 1968). Electrical stimulation of the caudate nucleus (Buchwald, Wyers, Okuma and Heuser, 1961), the preoptic area and the basal and medial amygdaloid nuclei (Clemente and Serman, 1963) has been shown to produce cortical slow wave activity and eventually sleep. Stimulation of the globus pallidus and internal capsule, too, have been reported to cause cortical spindling (Dieckmann, 1968). Comparable effects have also been produced by chemical stimulation. Acetylcholine, when applied to the preoptic area, was found to cause sleep, while increased alertness resulted from use of noradrenalin (Hernandez-Peon and Chavez-Ibarra, 1963). The previous stimulation and lesion results would suggest these may be excitatory and inhibitory transmitters respectively. More recent studies have also shown that caudate stimulation decreases the size of simultaneously recorded cortical evoked potentials to auditory

stimuli (La Grutta, Amato and Militillo, 1970), suggesting the animal is less responsive to external stimuli when this area is active. Thus, it is believed that there is an inhibitory system located rather diffusely within the forebrain which is antagonistic to the reticular activating system described first by Moruzzi and Magoun (1949). According to this interpretation, the conditioned inhibitory responses recorded in the previous study would represent an inhibition of this inhibitory system, and would functionally represent a transient increase in arousal of the animal during the corresponding interval. This, however, is an inference from the stimulation and lesion data only, as there are no previous reports of forebrain cellular activity correlated with the arousal state.

A second interpretation suggests that this response is more specific, being specifically related to positive reinforcement or food reward. Stimulation of the medial forebrain bundle (MFB) has been found to be positively reinforcing (Olds, Travis and Schwing, 1960) and to result in stimulus-bound eating (Hoebel and Teitelbaum, 1962). It is known that the MFB sends ascending fibers which end upon cells in the forebrain region. Ito (in press) showed reciprocal relations between lateral hypothalamic cells and preoptic area cells in the MFB. Rewarding brain stimulation in posterior hypothalamus caused inhibition of the lateral hypothalamic cells nearer to the point of stimulation and excitation of the more distant ones. Hamburg (1971) showed that the nearer group in hypothalamus was suppressed

during feeding but possibly excited during food anticipation. In Experiment I, the CS+ caused food anticipations and therefore might excite the hypothalamic group and this might be correlated with inhibition in the preoptic area elements. Travis, Hooten and Sparks (1968) described inhibitory responses of units in globus pallidus related to certain aspects of food acquisition and hypothesized that they signaled attainment of food reward. Berger, Wise and Stein (1971) showed that by injecting noradrenalin (a supposed inhibitory transmitter) in the ventricular region near the preoptic area, they could cause aphagic rats to eat. All of these data permit the guess that inhibition in the preoptic area could be associated either with the period just prior to eating (the anticipatory period) or with the eating period itself.

The present experiment was designed to differentiate between these two interpretations by alternately conditioning the animal to food and shock. If conditioned inhibitory responses were found regardless of the nature of the unconditioned stimulus, the first arousal hypothesis would be supported. If they were present only when food was the unconditioned stimulus, the food-reward hypothesis would be strengthened.

METHOD

Subjects

The rats were similar to those described in the initial experiment and included a small subset (some whose first conditioning experience was with food as the unconditioned stimulus) of those run in Experiment 1. In addition to the chronic electrodes, they were also outfitted with shoulder electrodes made of 250 μ diameter nichrome wire, via which an aversive shock could be delivered to the animal. The shock was a 60 c.p.s. alternating current of approximately 2 ma. in intensity and one-third second duration. The shock electrodes were also threaded through the animal's teflon plaque, and the stimulus delivered via the commutator cable, such that its introduction into the experiment in no way impaired the freedom of movement of the animal.

Procedure

The first experimental night consisted of the pseudoconditioning procedure as in Experiment 1, in which the unconditioned stimulus was that used on the first conditioning night. The animals were then alternately conditioned to food and shock over the next three nights. The second night approximately one-half of the animals were run with shock as the unconditioned stimulus, the other half with food as the unconditioned stimulus. The third night, the unconditioned stimulus

was changed such that those animals previously conditioned to food, now experienced the shock as UCS and vice versa. The conditioning stimulus was also reversed such that one tone was always conditioned to food, the second, to shock. This was done to ensure that the animals would learn to differentiate between the two responses with as little difficulty as possible. In general, the responses to the two conditioning stimuli during pseudoconditioning were similar, such that the learned responses to the two unconditioned stimuli were evaluated against comparable baselines. The fourth conditioning night was a repeat of the second. Each conditioning sequence was preceded by two hours of the pseudoconditioning procedure which effectively served as an extinction of the response learned in the previous session. When the shock was used as the UCS, the animal was fed during the preceding day. Data collection and analysis were the same as described in Experiment 1. Conditioned responses on Days 2 and 4 were compared to Day 3 to determine whether the response of the unit was specific to either experimental procedure.

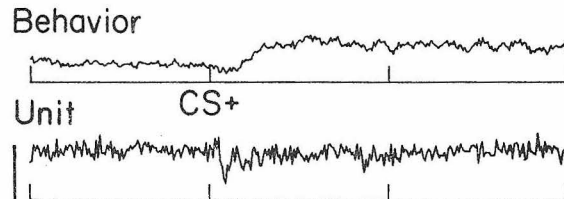
RESULTS

Two units showing inhibitory conditioned responses were tested over 4 sessions, one in the food-shock-food sequence, the second in a shock-food-shock series. One was a preoptic unit, the second from hypothalamus proper. In each case there was a significant inhibitory conditioned response whether the UCS was food or shock. The series of these responses for one of these units, the preoptic unit, is illustrated in Fig. 15. The quarter-second deviation scores for these responses are shown in Fig. 16. Although there appears to be greater inhibition in the conditioned food response than in the conditioned response to shock, there is yet considerable similarity between the responses as regards their latency and duration.

Figure 15.

Average trial histograms for preoptic unit and behavior to CS+ over four consecutive days. The first two pair of traces are from the first pseudoconditioning day, in which one can see habituation of the inhibitory response by the second one-half of the session. On the following three days, there is a conditioned inhibitory response to the CS+ whether the UCS is food or shock, and whether the animal's movement is increased or decreased. On the second conditioning day, the third second should be disregarded as it contains a stimulus artifact resulting from the shock. The vertical bar to the upper left unit trace represents a firing probability of .20 per 10 msec. period. The computer drawings to the right of each day's histogram represent sample drawings of the unit from which recordings were made on that day.

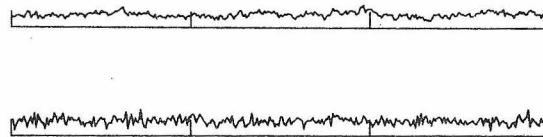
Pseudoconditioning- trials 1-150



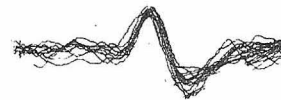
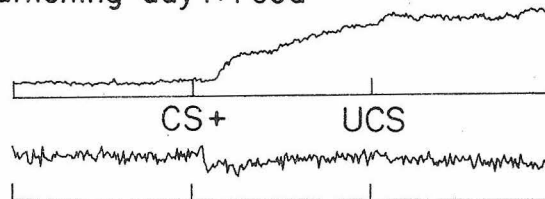
Sample
Computer Drawings
of Unit



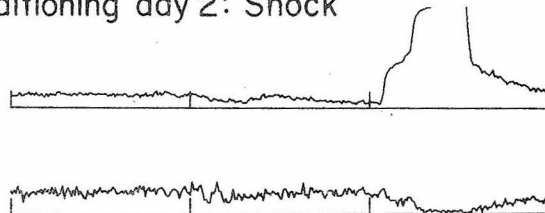
Pseudoconditioning- trials 151-300



Conditioning day 1: Food



Conditioning day 2: Shock



Conditioning day 3: Food

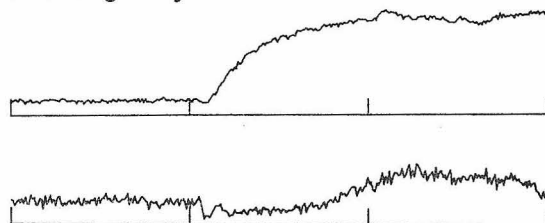
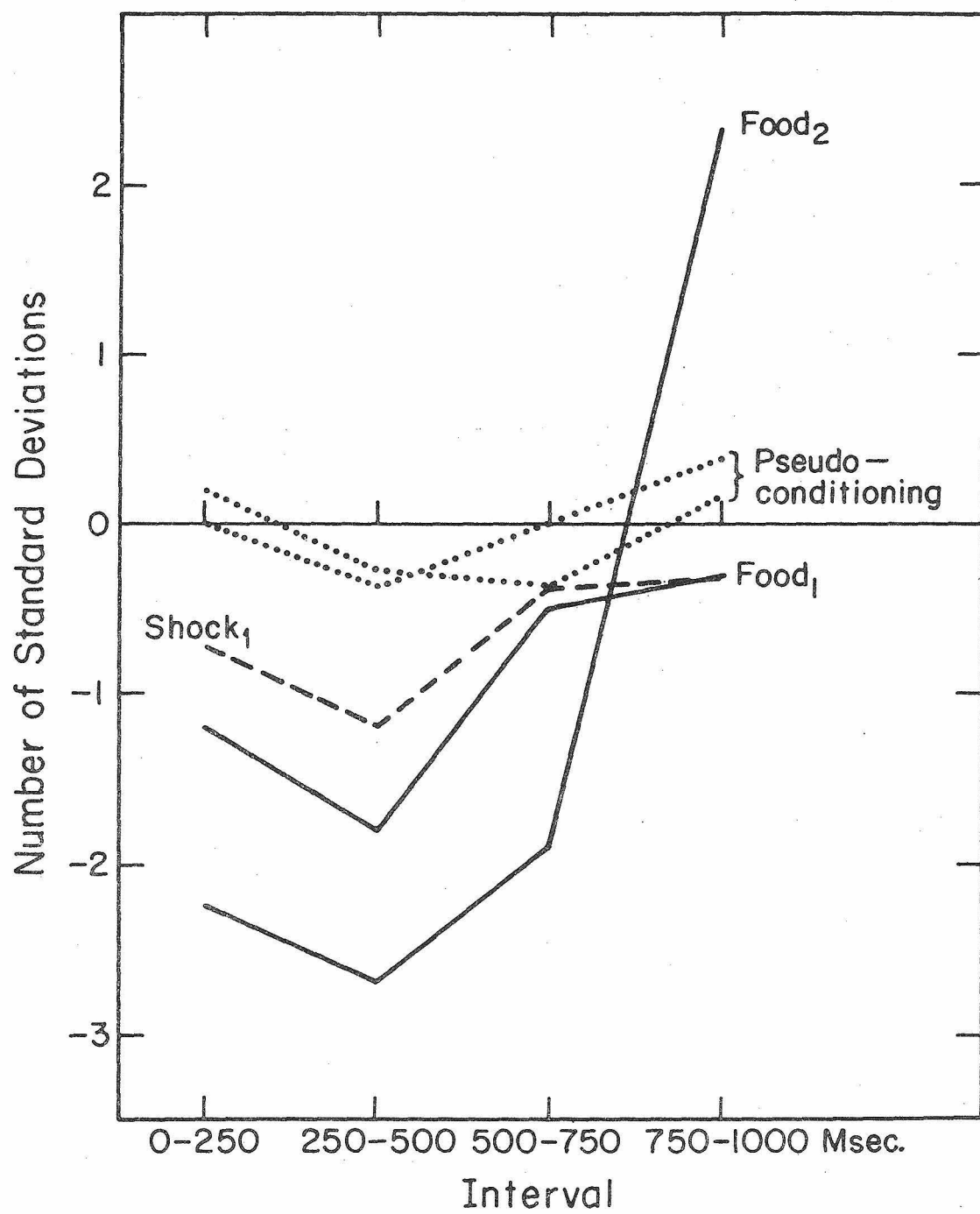


Figure 16.

Quarter-second deviation scores for unit response to CS+ over four days, as shown in histograms of Fig. 16. The dotted lines represent the deviation scores of the unit response to each of the tones alone during the second half of the pseudoconditioning trials. As can be seen, they show no significant unconditioned response, and differ little, and therefore serve as comparable baselines against which to measure the conditioned responses to food and shock, for which the tone stimuli were reversed.



DISCUSSION

Conditioned inhibitory responses were found regardless of whether the UCS used was food or shock. The results of this experiment therefore support the arousal interpretation of the responses. One might argue, however, that there was still sufficient difference between these responses, for example, in amplitude, to encode the nature of the UCS to follow. This is a possibility not provided for within the framework presented here. The question could be resolved, perhaps, if units were so tested in sufficient numbers, and consistency of such encoding be found. However, it is difficult or impossible to match food and shock for motivational intensity. What appears most striking is the similarity of the inhibitory response, in the two situations, even though both the motivation and the conditioned behavioral response were different.

FURTHER DISCUSSION

These inhibitory responses, then, appear to reflect the temporary release from the effect of a forebrain system which acts normally to inhibit the arousal activity of the animal. The responses, in general, did not have very short latencies; there was one as early as 40 msec., but most showed latencies of 100 msec. or greater. They were also generally transient, terminating before the end of the CS-UCS interval. In a few cases, there also appeared to be a second short inhibitory response of the units following the feeder discharge, but this was not statistically measured.

An interesting set of correlations appeared between the forebrain inhibition in the present study and "cortical disinhibition" which was studied by Susz, Buchwald, Hull and Smith (1971). They examined the effects of an auditory CS on cortical inhibition produced by background stimulation of caudate and measured by suppression of cortical unit rate. There was a disinhibition of cortex within 80-150 msec. following stimulus onset, similar to the latency of forebrain inhibition in the present study. The disinhibition did not appear during consummatory milk-drinking as the inhibition recorded here disappeared by the end of the CS-UCS interval (when the consummatory response was about to start). The amount of the cortical disinhibition seemed proportional to the amount of orienting required, as the inhibition recorded here was greater in the case of the food UCS (which required orientation as the shoulder shock UCS did not). These data

made it appear that forebrain activity might be the mediator of cortical inhibition.

This view comports with other views that a forebrain inhibitory system acts in opposition to the reticular activating system in its effects on cortical and behavioral arousal. Stimulation in the forebrain system can cause spindles (considered characteristic of a drowsy condition). Stimulation of the reticular activating system can counteract this inhibitory effect of forebrain stimulation if of sufficient intensity and vice versa (Buchwald, Wyers, Lauprecht and Heuser, 1961; Clemente and Serman, 1963), but the point of interaction of the two systems is not completely clear. Clemente and Serman (1967) and Heuser, Buchwald and Wyers (1961) believe the interaction is at the thalamic level, that is, that the forebrain stimulation suppresses and the reticular activating system excites thalamic structures controlling cortex. Susz et. al. (1971) and Hull, Buchwald and Vieth (1967) also implied a thalamic site of the interaction. However, thalamic activity studied by the present methods (Disterhoft and Olds, in press) did not show a distinctive inhibitory process. The present results therefore seem to suggest that the reticular activating system may act on the forebrain cells to inhibit them, which would then result perhaps via a thalamic pathway, in cortical disinhibition. Nauta and Kuypers (1958) and Scheibel and Scheibel (1967) have described axons which distribute into the basal forebrain area directly from reticular formation, via

which such inhibition of the forebrain could be effected.

This possibility could be further tested using an acute preparation to determine whether forebrain cells might be found that were turned off by reticular formation stimulation, were excited antidromically by stimulation of midline thalamus, and whether the anticipated changes in cortical EEG would occur.

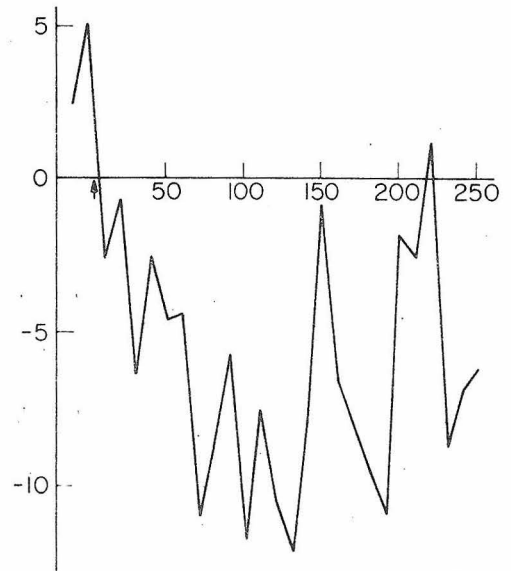
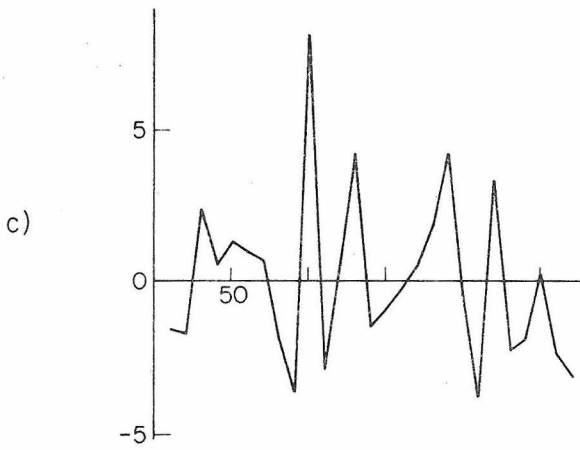
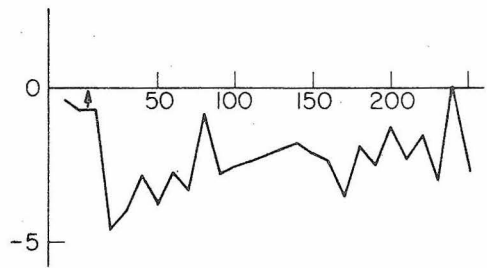
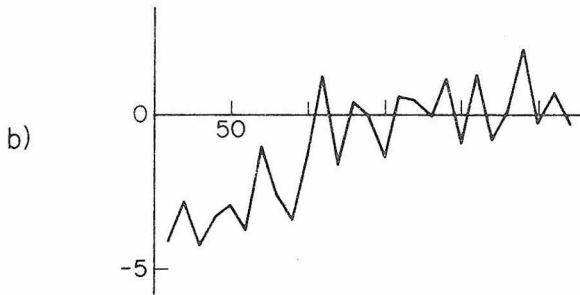
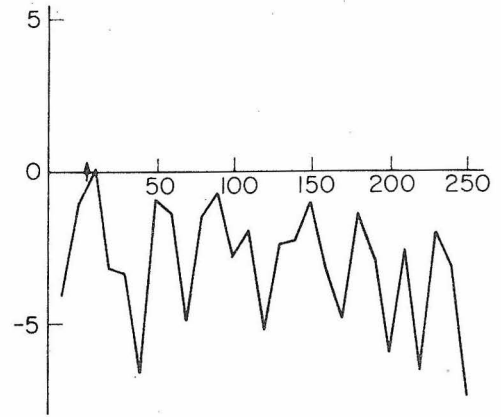
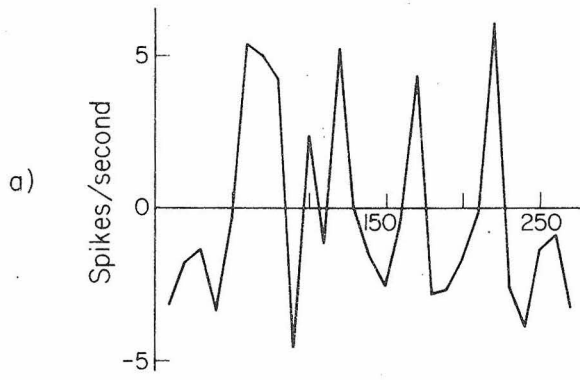
When the emergence of these inhibitory responses was examined for the group over trials, the "learning effect" was dwarfed by a slow, rhythmic variation in responses to the CS (see Fig. 13). When the individual curves were examined separately, there were 3 dominant patterns (see Fig. 17). The largest number of units (4 out of 8 cases) showed a pattern of periodic increases and decreases, with about 7 cycles a day (see Fig. 17a). This was comparable to the mean curve. The curve was in many respects similar on Day 1 (pseudoconditioning) and Day 2 (conditioning). However, there was a substantial shift in the inhibitory direction on Day 2. In spite of this substantial shift, there was no apparent trend on Day 2 (that is, no "learning curve"). Actually both Day 1 and Day 2 started with substantial inhibitory responses (when the stimulus was novel); on Day 1 this inhibition was reversed to excitation during the peak of each cycle; on Day 2 this did not happen. Another two units showed a clearer habituation of the inhibitory response during pseudoconditioning, and an apparent restoration of this response during conditioning (see Fig. 17b). The remaining two units showed cycles

Figure 17.

Examples of three patterns of response to CS+ over trials of inhibitory-response units during pseudoconditioning and conditioning. a), b) and c) are explained fully in text. Again, all curves represent response minus background. The arrow on the abscissa of conditioning day indicates the beginning of correlation of CS+ and feeder on that day.

PSEUDOCONDITIONING

CONDITIONING



as in the first group, but aside from that they appeared relatively unchanged on Day 1 and developed a substantial conditioned inhibitory response on Day 2 (see Fig. 17c). In most cases there is some ground for arguing for a waxing and waning effect (with about a 2-hour cycle) on both nights with a superimposed conditioning effect on Day 2. In most cases also there was an inhibitory response at first on Day 1.

It did seem interesting that the duration of the cycles (of possible responsiveness to the stimulus) within the CS curves was in the range of duration of cycles of activity measured for the laboratory rat. Richter (1927), measuring activity of rats in a stabilimeter cage, measured a cycle of two hours for rats to whom food was not available, but a cycle of four hours when food was available ad. lib. In the present experiment food was regularly presented, but not at the rat's discretion, an intermediate condition.

If these patterns of response of inhibitory units are real, then such units may be related to arousal functions in two ways - one associated with an internal rhythm of the animal, and a second responsive to novel or motivationally important stimuli. It should be mentioned, however, that there was no indication of comparable variability over time in performance of the conditioned behavioral response or in the background rates of these units.

Finally, units often had biphasic responses; that is, they showed

inhibitory conditioned responses in one part of the CS-UCS interval, but excitatory responses in another part of the same interval. This patterning suggests an interesting possibility. Since the cortical spindling response to forebrain stimulation is produced only by stimulation in a certain low frequency range, and very high frequency stimulation very often produces excitatory motor effects, it has been suggested there are in fact two populations of cells mediating these two effects, sensitive to low and high frequency electrical stimulation respectively (Stevens, Kim and MacLean, 1961). The present results suggest the same unit may somehow participate in both effects. This would be suggested only if units recorded with the present methods represented a homogeneous group of cells; the alternative would be that a functionally heterogeneous "unit" was observed, with different individual cells each performing only one function.

In summary, these conditioned inhibitory responses appear to reflect possible cellular correlates of an inhibitory forebrain system previously inferred on the basis of stimulation and lesion studies alone. They suggest that the point of interaction between the reticular activating system and the forebrain inhibitory system may be directly upon the forebrain cells, a possibility which could be further tested using a combination of stimulation and recording techniques. Further, the opportunity to obtain chronic recordings from these cells has shed some light on the possible functioning of such cells in a normal behavioral situation.

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